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Towards less formal arms control?

What will happen to the cause of arms control if the Salt II treaty is never ratified? More than a year has now passed since the treaty, the product of seven years of negotiation, was signed by the Soviet Union and the United States. Since then, the treaty has languished in Washington, awaiting a fair wind in the US Senate where, like any other treaty, Salt II has to command a two-to-one majority before it can be ratified. The time has never been right. Faced with the prospect that the treaty would be thrown out by the Senate a year ago, the Administration let proceedings hang fire while lobbying for the support of individual senators. The Soviet intervention in Afghanistan was, ironically, a godsend for the treaty. The Administration was able to put the treaty on ice and thus to avoid the near-certainty of outright defeat. So far as can be told, the Soviet Union has been sympathetic. While Soviet spokesmen repeatedly complain at the delay in ratifying the treaty, none of these complaints has been coupled with the obvious threat that the treaty will be abandoned if the Senate does not quickly give its consent. Indeed Mr Brezhnev has gone so far as to suggest that Soviet ratification is by no means automatic, thus giving the impression that the US Senate is not the only cause of delay. Yet a treaty has no legal force if it is not ratified. As things are, either of the signatory governments could repudiate Salt II without turning a hair — and there are some in the United States who guess that precisely this will happen if Mr Ronald Reagan wins the election next month. What in the circumstances, should be done?

The first need is not, at this stage, to abandon hope. The outcome of the presidential election will be important but not decisive. Mr Carter's re-election would not by itself assure quick passage for the treaty; the re-election of the supporters of the treaty in the Senate — people like Senator Hart — could be more influential. On the other hand, if Mr Reagan is president of the United States in the new year, it is unlikely that he will act as intemperately as he was suggesting when he was still campaigning for the Republican nomination. Like all his predecessors, he would find that there is precious little room for manoeuvre. Given the widespread recognition in the United States that a continuing relationship with the Soviet Union is an indispensable component of national security, and public knowledge of the importance of Salt II in Soviet eyes, no new president is going to tear up the treaty. What might, with advantage, be done is to explore the possibility of modifying the treaty and, in the process, of improving it. Thus the expected five-year lifetime of Salt II is absurdly short. If it takes a decade or more to develop new strategic weapons, what sense can there be in a treaty which limits the numbers of missiles deployed only for a period of five years? Talks about improvement would help to get both Mr Carter and Mr Reagan off the hooks on which they are now impaled.

Even so, Salt II may remain in limbo. What would be the consequences for other initiatives in arms control? The most obvious dangers lie outside the strictly bilateral relationship of the Soviet Union and the United States. At the beginning of September, the signatories of the Non-Proliferation Treaty made loud complaints about the failure of the super-powers to ratify Salt II (see *Nature*, 11 September) — and even demanded that, pending ratification, the two governments should declare their intention of behaving as if ratification were a fact. (For the United States, such a course would be unconstitutional and, in any case, is prohibited by the US arms control legislation.) Most probably, the super-powers will be able to hold this ring. A more serious difficulty is that, hitherto, other items on this still thin agenda of

arms control have been dependent on the conclusion of Salt II. This is why it is a hopeful sign that the Soviet Union and the United States seem willing to start talking about limitations of nuclear weapons in Europe — a process which West Germany in particular is keen to see begun. The main lesson seems to be that the frustrations of Salt II seems not to imply that other negotiations on arms control must be brought to a halt.

But what to talk about? In what forum? And what, in any case, would be the objectives? The negotiations about a comprehensive test-ban treaty are the most advanced, but the United States administration cannot realistically think of signing such a treaty until after the new Senate has had its say about Salt II. There is also an urgent need, not yet realized, for exploratory talks on the deployment of chemical weapons. After a ten-year moratorium on the manufacture of chemical weapons in the United States, the Department of Defense is now embarking on a programme for the manufacture of binary chemical weapons — devices in which two chemical components of a nerve gas are mixed together only when the shell or bomb that carries them is detonated. The decision has been influenced by the opinion that the Soviet Union is redeploying chemical weapons along the European border, but the evidence is only thin. Chemical weapons may not be central to strategic arms control, but they are powerfully symbolic. No harm but only good would come from bilateral negotiations on this subject.

Intermediate-range nuclear weapons, principally in Europe, are a harder problem for two different sets of reasons — political and technical. First, there is a serious dispute among the interested parties about the list of participants for Salt III. The Soviet Union has a simple line — negotiations about nuclear weapons in Europe must be conducted bilaterally between itself and the United States. No doubt it would be an embarrassment if the other members of the Warsaw Pact were allowed to have their say on matters which have hitherto been decided unilaterally by Soviet strategic planners. In the West, however, multilateralism is in the air. Nobody in Western Europe will be satisfied with a bilateral deal on non-strategic nuclear weapons between two super-powers which would not necessarily be in the front line if the weapons concerned were ever used in anger. Such a deal, arrived at without the consent of Western European governments, would probably turn Western Europe into a Gaullist citadel, more determined than at present to paddle its own canoe, both in planning for defence and in negotiations. Although the Soviet Union and the United States have this week (in Vienna) begun talking about the problem of the so-called Euromissiles, their talks will be mere chats if they do not devise some way of securing the open participation and consent of at least the Western European governments.

But what will these talks accomplish? Salt II was successfully negotiated only because limitations of long-range strategic weapons could be defined arithmetically. Each partner in the negotiations appears to be content with a fixed number of long-range missiles, based in silos and in submarines. (No doubt the knowledge that the quantity of nuclear explosives permitted is vastly greater than that needed for virtually total destruction of the other side has helped to make these limits acceptable.) The technical obstacles to a corresponding agreement on Euromissiles are more serious. What on one side is equivalent to what on the other? Will it be possible to distinguish between strategic and tactical delivery systems? What are the consequences of the geography of Europe? At this stage, nobody can tell whether



these obstacles can be surmounted. In the end, an agreement on Euromissiles (if there is to be one) may have to be quite different in character from Salt II. It helps that the Soviet Union and the United States have at least begun flirting with the problems. It may not be a productive negotiation, but it cannot fail to be educative.

The outcome will not, however, be a formal treaty unless Salt II is ratified. The Soviet Union has made that plain. What, in the circumstances, can be the outcome of the Vienna talks (which are likely to drag on for years to come — unless Mr Reagan is ever in a position foolishly to put an end to them)? If the two sides merely understand each other's views of strategic weapons more fully, that will be worthwhile. In retrospect, it is clear that the negotiations ten years ago about anti-ballistic missiles helped to persuade both sides that their deployment would be counter-

productive, with the result that Salt I (with its limitation on anti-ballistic missiles) was something of a formality. Thus there is something in the view that in arms control negotiations the process may be as valuable as the product. This recognition has tempted some to go further and to argue that, in arms control, tacit understandings between the major powers are in some ways preferable to formal treaties. Mr McGeorge Bundy, adviser on national security to Presidents Kennedy and Johnson, was making this case only last week at a seminar at Massachusetts Institute of Technology. That tacit understandings might be more easily agreed, and even more ambitious, is beyond dispute. The snag is that almost by definition they cannot be policed or relied upon internationally. But if Salt II remains in limbo, or if Mr Reagan is elected and does his worst, they may be the best hope for arms control in the 1980s.

The space agency's neglect of science

The death last week on a climbing expedition in the Himalayas of Dr Thomas A. Mutch, associate administrator of the US National Aeronautics and Space Administration (NASA) will be widely mourned. In the past year, since his move from Brown University, Dr Mutch has helped to give NASA's support of scientific research a sense of greater civility than has often been the case in the past.

Sadly, however, Dr Mutch's death will inevitably prompt further questions about the adequacy of NASA's general strategy on scientific research. From the beginning, the agency has lived in an uneasy and ambivalent relationship with the scientific community. For many people and groups, of course, it has been a source of unaccustomed and even unexpected largesse. But there has also been a constant grumble, from universities and other places where space vehicles are thought of as a means to an end, that the agency takes too short a view of its contribution to scientific research. There is a large stock of anecdotal evidence that it is far easier to wring from NASA quite large sums of money for putting things in spacecraft of various kinds than for the frequently less expensive task of making good use of the data which are eventually collected. What with one thing and another, but especially because of the way in which getting the space shuttle built is eating into NASA's budget, there is a nasty fear that science will be a conspicuous casualty of the years ahead.

The symptoms of present discontents are easily listed. The most evident is the prospect that, in the immediate future, there will be a dearth of Earth satellites and other spacecraft whose purposes are primarily scientific. Each interested party has a particular sense of grievance. Some most of all regret that projects like that to send a spacecraft to Halley's comet (due back within shooting range in 1986) may not get the funds they need in next year's budget. Others weep about the impending premature demise of the Einstein X-ray observatory, and the near certainty that there will not be a comparable instrument to replace it within this decade. The device known as a solar polar orbiter, the chief objective of which is to make measurements of the solar wind well away from the Sun's equator, survived only by the narrowest squeak the budgetary process earlier this year, largely because of the intervention of Dr Frank Press, the President's Science Advisor, and is probably now safe. It is also likely that the Large Space Telescope, the instrument confidently planned as the next big step forward in visual astronomy, will not now see its way into orbit until the second half of the 1980s. Some gloomy folk even fear that the cost of the telescope (likely to be more like \$1,000 million than the original estimate of half as much) will itself turn out to be an internal pressure within the NASA budget.

The prospect that there will be a shortage of hardware in the remainder of this decade is, however, less alarming than that which faces the groups which have grown up in recent years and which are primarily concerned with the design of instruments carried on spacecraft and the interpretation of the results which they produce. NASA has rightly taken the view, since its earliest

times, that one of its objectives should be the encouragement of research in universities and elsewhere. On several occasions, as for example when there was more Moon rock to be cut into specimens than there were suitably equipped investigators, NASA has glad-handed its dependants in an almost lavish way. Yet even where that huge (and expensive) harvest of material is concerned, the first flush of enthusiasm has not been matched by sustained effort. Although the Einstein satellite is reckoned in its truncated life to have produced so much data that those now working with it could be kept busy for six or seven years, there is a suspicion that the funds needed to make the most even of what exists will not be forthcoming. Certainly the data needed to make the most of the data which exist — and which, for example, may be especially important in making sense of variable X-ray sources — will not now be available until the next decade. One result is, that some of those who flocked into X-ray astronomy only a few years ago, when the Einstein satellite was being planned, are now flocking back again to their earlier fields of interest — wiser, perhaps, but also more cynical. People understandably have the sense that NASA as a whole is more interested in the ballyhoo that attends the launching and the operation of a major scientific satellite than in the more tedious but necessarily continuing task of making sure that the mission has been worthwhile.

To those responsible for NASA as a whole, views such as these may seem but evidence of base ingratitude on the eve of the close encounter of Voyager I with the system of Saturn and its rings, planned for 12 November. Between now and then, increasingly spectacular photographs of the planet and its satellites will no doubt be increasingly frequent in the daily newspapers. And in any case, the administrators will say, there is Voyager II, following six months behind, still to come. What on earth can be the complaint? The simple answer is that even at this late stage there is no confidence that there will be funds to support the continuing work of the groups now concerned with harvesting data from these encounters. People who have cut their teeth on these endeavours will find themselves looking for jobs elsewhere.

For NASA, the problem is one not merely of budgets but of procedures — or that, at least, is how the agency should set about putting its house in order. It is of course entirely understandable that an agency committed, wisely or otherwise, to a novel project such as the space shuttle should be unable to keep every programme intact when the costs turn out to be far greater than originally foreseen. It does not, however, follow that the agency has no choice but to sit back and accept the fate which Congress and the Office of Management and Budget decree when things go wrong. And if the need to accommodate the cost of the shuttle within the limits laid down by the Administration means that much valuable scientific data is literally wasted, NASA has not merely a right but a duty to tell its paymasters that money is being wasted. In short, if NASA shares the view that science is being needlessly neglected, it should say so. Otherwise, it should let the scientific community know more clearly where it stands.

Another route to fusion to be explored

US looks hard at inertial confinement

Washington

The Department of Energy (DoE) is proposing to set up what one official describes as a "bluest of blue ribbon" review committee to look at the prospects of developing inertial-confinement fusion (ICF) techniques as a commercial source of nuclear power.

The decision to take a hard look at ICF comes close on the heels of a new bill signed into law by President Carter two weeks ago pledging support for a substantially expanded programme of research into magnetic confinement fusion, at present the front runner in the fusion race.

The Administration has been persuaded to back the Magnetic Fusion Bill by a report from the DoE's Energy Research Advisory Board. This says that results using magnetic confinement techniques at, for example, Princeton University's Plasma Physics Laboratory, are sufficiently impressive to justify the next stage of the development programme.

The bill proposes setting up a National Magnetic Fusion Engineering Center to look at the technical problems involved in scaling up laboratory experiments. It also requires DoE to produce a five-year programme for magnetic fusion detailing budget and research schedules.

The same committee, chaired by Dr Solomon Buchsbaum of Bell Laboratories, has now been asked to look at the progress and prospects of ICF. DoE's proposal is contained in a letter from Energy Secretary Mr Charles Duncan to the Office of Management and Budget, accompanying the department's budget request for the fiscal year 1982.

Department officials feel that, largely as the result of significant advances made during the past year or so, research on the civilian applications of ICF has progressed sufficiently to make it possible to evaluate the relative merits of different systems now under development.

Research on ICF has also been given a boost by a recent DoE decision to start declassifying previously secret information on high-energy X rays produced by the explosion of deuterium-tritium pellets — the source of fusion energy.

Three weeks ago, the department reported that it had decided that the following statement should no longer be considered a military secret: "In some ICF targets, radiation from the conversion of focused energy (such as laser or particle

beam) can be contained and used to transfer energy to compress and ignite a physically separate component containing nuclear fuel".

DoE officials play down the impact of classification on the civilian programme, insisting that this has not prevented its own scientists from weighing the relative merits of different approaches. University scientists, however, have long argued that classification has been a barrier to their research; designs for ion drivers, for example, have required access to details of pellet behaviour in order to optimize beam/pellet interaction. "This is the news we have been waiting for", said one person on hearing DoE's decision.

The main problem now facing the ICF research community is how to choose the best technology for moving on to the logical next stage, the construction of a facility to probe the engineering difficulties that may lie ahead.

There is a general consensus that for magnetic fusion the Russian Tokamak design is still clearly the front-runner, but several options remain for the drivers needed to produce the energy that ignites the pellet for ICF. These include gas lasers, glass lasers and particle accelerators using light and heavy ions.

For the medium term, the best prospects seem to lie with either glass lasers or light-ion accelerators. Support for the former, in particular, has been increased by recent results from the University of Rochester, demonstrating that halving the wavelength of the light produces an ultraviolet laser beam that interacts much more efficiently

with the pellet than does an infrared laser.

Dr Moshe Lubin, director of Rochester's Laser Energetic Laboratory, said last week that it was now possible to reach an efficiency of at least 80 per cent in converting the wavelength of the laser beam. This raises the possibility that crucial scaling-up experiments could be carried out much sooner than expected.

Further ahead, both glass lasers and light ions could face major difficulties. The lasers are difficult to cool, and it might therefore be hard to achieve the high repetition rates necessary for the large-scale production of power. Light ions, although effective at close range, are difficult to focus accurately.

Here, two other options may come into their own — krypton fluoride lasers, now being developed at the University of California's Lawrence Livermore Laboratory, and heavy-ion beams, at present being squeezed for funding by a congressional preference for glass lasers with potential military applications, but eagerly supported by nuclear physicists as an extension of particle accelerator research.

DoE officials are more sceptical, suggesting that some of the claims of nuclear physicists have been over-optimistic and partly motivated by inter-laboratory competition for funds. Nevertheless, they admit that heavy ions are a serious contender for eventual commercialization, and hope that the new review committee will throw some light on where research priorities should lie.

David Dickson

Canada's uranium with strings attached

There is a prospect of a dispute between Canada and Euratom over uranium supplies. Canada is prepared to stop uranium supplies to Europe if Euratom states fail to comply with Canadian non-proliferation policy. But Euratom "is not going to surrender", according to Euratom and Canadian officials.

The confrontation will come to a head later this year, when an interim arrangement reached in January 1978 expires. And since Australia is taking a similar stance to that of Canada and the position of the United States — the other potentially large uranium supplier in the 1980s and 1990s — is clouded by the 1978 Non-Proliferation Act, uranium supply negotiations are becoming an increasingly sensitive element in the future growth of nuclear power.

Australia is furthest ahead in its negotiations with Europe. West Germany has a major share in the new Ranger mine, which will give the country 20,000 tonnes of uranium oxide in 1982–86 according to a recent agreement with the Australian

government — provided Australia secures satisfactory non-proliferation agreements with Euratom. (Euratom currently negotiates safeguards with suppliers for its nine members, and must "approve" any bilateral supply deals. But its supra-national authority is under strong challenge from France — a further complication.)

France also has a draft deal with Australia for the supply of 2,000 tonnes of uranium a year — a fifth of its estimated need in 1990 — but again that awaits an agreement between Australia and Euratom. The draft deal itself includes outline safeguards arrangements; but both sides have dodged giving them firm interpretation. Australia, mindful of the strong anti-nuclear lobby at home, has a public commitment (as does Canada) to ensure that none of its uranium is used for weapons building; so its policy is to require "prior consent" for re-transfers of its uranium outside Euratom states, for re-processing and for enrichment beyond 20

per cent. According to Australia, the draft deal with France is in accord with policy; but according to French statements it offers no restrictions on reprocessing.

French industry minister André Giraud said recently that "we have no intention of using a single gramme of Australian uranium for our military programme" but, challenged on safeguards, said "we can say exactly where our power plants are and how Australian uranium will be used".

Hoping that such statements will satisfy the Australians, Euratom negotiators expect to tie up an accord soon, thus clearing the tables for Canada. Euratom fears Canada most; the US position is too uncertain to consider yet.

Canada — which already supplies 50 per cent of Britain's uranium (the rest comes from Namibia) — has the experience of India's 1974 nuclear test, which used Canadian uranium, to keep her resolution firm. The Indian test raised a storm of public protest; and although non-proliferation is no longer an election issue, it could become one if a similar incident occurred with Canadian involvement. And then uranium export might be halted. Thus, Canadian officials argue, it is in the interests of security of supply that Canada requires clear controls over its uranium.

Canada's position is ostensibly the same as Australia's — requiring prior consent for reprocessing, re-transfer and high enrichment. But, say Euratom states, such an arrangement would interfere with national sovereignty, complicate fuel management and give Canada unfair advance warning of impending international commercial deals. Canada, after all, with its fuel-efficient Candu reactors and heavy-water plants, is in the nuclear business as a whole, and not just in uranium exporting.

In the end, everything depends on exactly what is meant by "prior consent". Euratom could be expected to object strongly if it means that each sensitive step in the fuel cycle must first be cleared with Canada: for since fuel streams are inevitably mixed when fuel elements are fabricated and fuel enriched or reprocessed, any reporting of movements to Canada would involve reporting — and clearing — all steps in the cycle. France has stated openly that it would accept no restrictions.

On the other hand, Canada has indicated that it is prepared to interpret prior consent "more broadly". For example, Canada might accept an agreement which stated that its uranium was to be used only for energy, and specified the facilities at which the uranium was to be reprocessed. Canadian experts would still have to be satisfied that Euratom's uranium book-keeping was effective, and that Canadian uranium could be "tracked" through the system.

And Euratom need be in no doubt about the firmness of Canada's commitment. "We stopped all uranium shipments in

1977 and we can do it again" said a Canadian nuclear official last week. Canada has already cancelled deals with India, Pakistan, and recently Switzerland — which objected to "prior consent". To cancel arrangements with Euratom would be more expensive (the UK needs contracts for another 2,000 tonnes of uranium per year by 1990, and France another 8,000 tonnes worth some \$500 million dollars a year) but that appears not to frighten anyone in Canadian government. It does, however, frighten Europe. **Robert Walgate**

Swedish guidelines

Cloning committees

Stockholm

The regulation of hybrid-DNA research in Sweden is in a mess. Delegates to a recent microbiological conference at Umeå bemoaned the bureaucratic confusion foisted on them by a new regulatory system; and one firm has given up and is moving its activities abroad.

Since 1 January this year, any researcher wanting to work with hybrid DNA has been legally obliged to apply for permission under two existing laws: the Occupational Health and Safety Act (Arbetsmiljölagen) and the Law on the Protection of the Environment (Miljöskyddslagen). The bodies that deal with the applications are the National Board of Occupational Safety and Health (NBOSH: Arbetsarkyddsstyrelsen) and the Franchise Board for Environmental Protection (FBEP: Koncessionsnämnden för miljöskydd), respectively. Both bodies are served by a Recombinant DNA Advisory Committee, set up in January under NBOSH. The committee's main task is risk classification. It has 17 members excluding its chairman (the director-general of NBOSH) and vice-chairman: four scientists, four members of parliament, five delegates from relevant authorities (National Board of Health and Welfare, Natural Science Research Council, etc.), three trades union representatives and one representative of industry and employers.

Both NBOSH and FBEP refer applications to the committee, which in turn passes them on to its working group on risk classification. If the application came from NBOSH, the working group classifies it and decides whether or not it is for new research (under the Occupational Health and Safety Act, only applications for new research have to be approved). The working group then makes its recommendation to the committee, which passes it on to NBOSH. The trouble with this is that nobody is quite sure what "new" research is. "It is difficult to interpret the meaning of this law", says Gustaf Brunius, who is in charge of DNA questions at NBOSH. "But *E. coli* K12 is a system that has been very thoroughly investigated, and if an application involves

this system it is considered unnecessary to get permission for it unless the DNA sequences for toxins."

The procedure is even more complicated when the application comes from FBEP. FBEP sends the application not only to the committee, but also to 14 other bodies (including local government and health authorities and the Environment Protection Board), and asks for their comments. When all of them have replied — which takes months — FBEP holds a public meeting at which the application is publicly made and the various bodies which wish to comment publicly do so. FBEP then writes a judgement.

University researchers say they have not received any information about this system, and that they do not know how to apply. In practice, they are continuing their research without applying for permission at all, which technically makes criminals of them all. Only one firm has so far applied, and it is holding up its experience as an example of bureaucratic ineptitude. KabiGen which, in conjunction with Genentech Inc., owns the world rights for the production of human growth hormone, had applied for and been given permission to go ahead with developmental work under the old voluntary regulation system. When the new system was brought in, the firm made another application to use hybrid-DNA techniques to produce genes for human growth hormone, somatomedin B and human secretin. Six months later, permission was granted (in volumes of not more than 10 litres); but NBOSH specified P1 conditions, whereas FBEP specified P3. FBEP evidently did not take into account the revision in the National Institute of Health guidelines which occurred while it was considering the application. So KabiGen put in a new application to FBEP for permission to do the same work in P1 conditions. No decision has yet been made. The firm also applied again to NBOSH for a more general permission to do developmental work, and no decision has been made on that either. "We can do 10 litres here, but we want to do 400 litres", says KabiGen's research director, Dr Bertil Åberg. "It would take too long to get permission for that, and we'd lose too much money. We'll do it in England instead".

Some delegates to the Umeå conference, which was organized by the Swedish Microbiological Association (universities) and the Foundation for Biotechnical Research (industry), suggested that the researchers themselves should take some of the blame for the present chaos. "There must be experts on the Recombinant DNA Advisory Committee, but they need not be in the majority", said Staffan Normark, Professor of Microbiology at Umeå University. "But everyone taking part in the committee's decisions must know what the research is about. We in the field haven't told them; and perhaps we should."

Wendy Barnaby

Academic freedom

Promise for Poles

Poland is to introduce new legislation designed to meet the demands of the academic milieu for greater autonomy, according to Janusz Gorski, the Minister of Science, Higher Education and Technology. The first draft of the new bill should go before parliament in December, but many of its provisions regulating dealings between his ministry and the universities would come into force immediately. In particular, he said, the ministry would not appoint university rectors without prior consultation with the university senates.

Another development in the Polish academic world concerns the University of Warsaw, which has in the past couple of weeks had a sudden change of rector. The highly unpopular Dr Zygmunt Rybicki resigned shortly before the opening of the academic year, to be hastily replaced by historian Dr Henryk Samsonowicz. The significance of this change is not entirely clear — Polish academic life, like Polish life in general, is something of a melting pot at present. However, on 28 September, the university senate issued a statement which is, in effect, a blueprint for the restoration of academic autonomy.

The statement pays tribute to the new "self-governing" trade union movement, which has now spread to the university students, and makes a number of demands for the liberalization of society as a whole, including electoral and censorship reform. It then discusses the role of the universities which it says is, basically, to "carry out scientific research and to serve national culture and public education". Science, and the results of research, said the senate, should be implemented for the benefit of society: a commission of experts should be established, and science should be involved in decision-making at all levels.

The statement advocates open and unhampered discussion and demands a basic reform of the higher education system. The main points of such a reform, said the Warsaw senate, should be:

- (1) Self-governing and democratic status of universities and colleges of higher education, collegiate bodies, senates and faculties to decide on directions of research; secret elections of these bodies, with students and the working staff of the institution also represented; curricula to be decided in consultation with the relevant ministries.
- (2) Free access to publications and sources of scientific information.
- (3) Proper means and conditions of work guaranteed to students and staff.
- (4) Freedom for students to organize their own lives.
- (5) Academic appointments to be made on the basis of qualifications only.
- (6) The Supreme Scientific Council to possess all necessary competence for

shaping academic policy.

(7) The awarding of academic degrees to be the business of the collegiate bodies, faculties and senates, the Central Commission for Qualifications and the Council of State.

The question is, of course, how far these proposals will be implemented in the new legislation. Minister Gorski suggest that there will be some reforms in the awarding of higher degrees by the Central Commission for Qualifications.

However, the proposals announced for improvements in the student grant system have clearly met with less enthusiasm than Gorski had expected. The majority of Polish students, who have flocked to join the new "self-governing" student unions, appear more concerned with the lifting of censorship restrictions.

One good omen for the future, however: Miroslaw Chojewski, the young chemist dismissed in 1977 from the Swierk Nuclear Institute because of his membership of the then Workers' Defence Committee and on trial earlier this year for participation in the "independent" publishing movement, has been reinstated under the terms of the Gdansk accords. At least half of the Swierk staff, said Chojewski, personally endorsed his application for reinstatement.

Vera Rich

Endangered species

Mediterranean acts

The fifteen or so well-managed nature reserves around the Mediterranean are around ten times too few, the International Union for the Conservation of Nature (IUCN) is arguing at an intergovernmental meeting in Athens this week. Papers produced by IUCN for the meeting specify dozens of threatened species of mammals, amphibians, fish and plants.

The principal threat is tourism — expected to reach 200 million tourists a year by the year 2000 — with its demands for coastal hotels, marinas, and baubles such as coral, sponges and tortoiseshell.

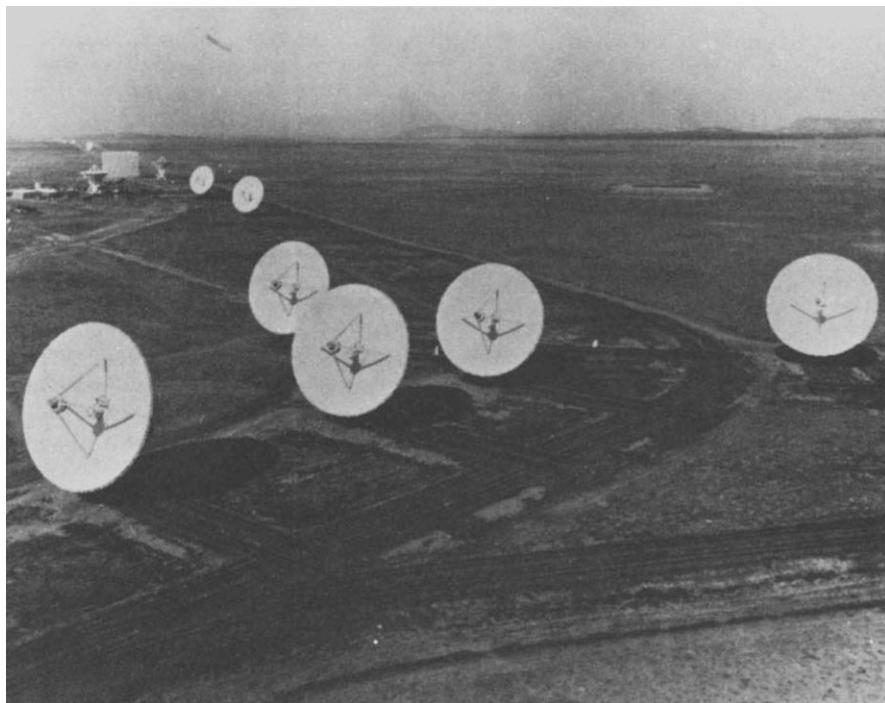
The most endangered species is the monk seal, *Monachus monachus*. Besides one colony on the coast of Mauritania in West Africa, there are just 1,000 monk seals around Turkey, Greece, Yugoslavia and Tunisia, and numbers are falling by a hundred or so each year. Numbers are now so low, and groups so dispersed, that the seals are threatened by restricted genetic diversity. The marine turtle is in similar danger, as the beaches where it lays its eggs are turned over to tourism. Other species at risk include the Iberian midwife toad, the Mediterranean spur-thighed tortoise, the

Biggest radio telescope in the world

The Very Large Array radio-interferometer was inaugurated last week in New Mexico by Dr Frank Press, the President's Science Advisor.

The array, which consists of twenty-seven steerable parabolic dishes 82 feet in diameter, has been brought progressively into operation since October 1975.

Individual dishes move along three double railway tracks at angles of 120 degrees — two of the tracks are 13 miles long and the third is more than 11 miles long. The array, operated as a national research facility by the National Science Foundation, is capable of greater angular resolution than any other instrument of its kind.



La Scuola wall lizard, the Israel painted frog, the smooth snake, the meadow viper and the spectacled salamander.

But it is not just a question of protecting pretty animals, according to Dr Pierre Hunkeler, the Swiss ecologist who organized the scientific side of the Athens meeting. Colonies of coastal plants and animals of great scientific interest need protection. Turkey and Greece have the richest coastal flora, followed closely by Morocco and Spain. Greece has 670 species of plants unique to the area (compared with Britain's 15) claim IUCN, and many of them are threatened.

Many of the ecosystems under attack are not yet well specified scientifically: in the wetlands, for example, like the protected Camargue (the Rhone delta), bird life is well understood but much less is known of the plants and water-dwelling animals. Ecologists and zoologists must have a chance to study these regions, argues Hunkeler, if only to give a baseline against which to measure the effects of pollution and development.

Representatives from ten Mediterranean nations had gathered in Athens by Monday: Greece and Turkey, France and Italy, Algeria, Libya, Tunisia, Israel, Yugoslavia and Malta were there. Representatives from Monaco and Cyprus were "on their way" from another meeting. The EEC was represented, but no sign had yet been seen of Spain, Morocco, Lebanon, Syria or Egypt.

The Athens meeting will avoid defining precise geographical regions to be protected. At that point, local political and development interests come into play, and costs and benefits must be worked out. If protected areas also mean protected fisheries, and increased amenities for the local populations and for tourists, then perhaps a deal can be reached which will satisfy both the ecologists and governments. But the Athens experts are leaving that to the politicians. **Robert Walgate.**

Electric cars

Win some, lose some

Washington

Plans for the development of a battery-driven electric car have taken one step forward and one step back. Scientists from the University of California's Lawrence Livermore Laboratory have announced the successful testing of a new aluminium-and-water battery which, they claim, can power cars for much greater distances than other batteries under development.

The announcement came only a few days after reports of tests by the Department of Energy (DoE) suggesting that Gulf and Western (G + W) may have been premature in some of the claims made for its new zinc-chloride battery, launched last June.

The Livermore results, presented at a meeting of the Electrochemical Society in Miami, are the products of research jointly

Plates in contact

Africa's slow movement towards Eurasia probably caused last Friday's 7.3-magnitude earthquake in El Asnam, Algeria, which destroyed 80 per cent of the city 20,000 killing perhaps people.

El Asnam, sits on a tectonic boundary between two plates — or so it appears, for the region is not seismically well characterized. The 1954, 6.7-magnitude earthquake in the same area, which killed 1,200, occurred before the world seismic monitoring network had been established. But measurements of magnetic anomalies between Africa and the United States, and between the United States and Eurasia, across the midatlantic ridge, indicate that the Africa-Eurasia boundary is closing.

The grisly event has one positive outcome: it will increase geological knowledge of the zone dramatically. Seismic stations can now pinpoint the epicentre to within 5 km, and by following the expected series of diminishing aftershocks (one of magnitude 6.2 occurred three hours after the main quake) determine the nature of the movement. This is expected to be a thrust, rather than strike-slip with Africa riding up over the Eurasian plate — the Atlas mountains themselves being part of the result.

Algerian authorities may not have a spot-on repeat of 1954; but they were criticized this week for not taking sufficient care to protect their buildings. The building codes for the El Asnam region call for stiffening to resist an extra 10 per cent weight static load, said Dr F.K. Farma of Imperial College, London, a civil engineer who made a study of the area. "But for that quake they needed 50 per cent".

Robert Walgate

sponsored by DoE and two large industrial corporations, Continental Group Inc. (working with the Lockheed Corporation) and the Diamond Shamrock Corporation.

The battery works by submersing an aluminium plate in a solution of sodium hydroxide. The reaction of the two with air produces an electric current and hydrazillite, an aluminium compound which subsequently crystallizes out so that the aluminium can be recovered.

Unlike more conventional storage batteries which require overnight charging, the new battery is claimed to need only to be refuelled with tap water every 250–300 miles. The aluminium plates would be replaced every 1,000–3,000 miles, but the operation should take only 15–30 minutes.

One drawback is the cost. Operating the new battery — including in particular the need to replace the aluminium plates at regular intervals — would cost the equivalent of between \$2 and \$3 a gallon, about twice the present US price.

However, this may still be competitive with gasoline made from coal — and it

would be as efficient to use coal for making the aluminium plates as for producing gasoline.

Meanwhile, a report in the *Wall Street Journal* that G + W is encountering technical problems in developing its zinc-chloride battery has raised questions about the extent to which the announcement was designed primarily as a publicity exercise. The newspaper quoted DoE reports that although G + W had claimed that the battery could power a standard car for 150 miles driving at 55 m.p.h., technical difficulties with charging the battery suggested that these figures were over-optimistic.

A spokesman for DoE, which has invested more than \$15 million in the G + W project, accepted last week that the development of the battery had encountered several technical difficulties, and that as a result some of the claims made by the company last June were probably premature. But he denied that the department had lost enthusiasm for the programme.

David Dickson

Nuclear wastes

Small disposals

Washington

Much to the relief of many east coast hospitals, universities and medical schools, the Nuclear Regulatory Commission (NRC) is proposing that liquid scintillation media used for detecting low levels of radioactivity in biological samples need no longer be buried in nuclear waste disposal sites.

At present, almost all scintillation media used in this way for biomedical research, as well as the carcasses of animals in which chemicals containing radioactive tracers have been studied, have to be transported in special drums 3,000 miles across the continent for disposal.

This expensive exercise — Harvard Medical School and its associated hospitals spent almost half a million dollars last year disposing of 3,000 drums in this way — has been necessary since the state of South Carolina announced a year ago that it was no longer prepared to accept low-level wastes from hospitals and research laboratories at its Barnwell storage site. Shortly afterwards, the nation's other two radioactive waste dumps at Richland in the state of Washington and at Beatty in Nevada announced that they too were closing their doors in protest at the poor way in which low level waste was being packaged and shipped.

Alarm spread quickly through the medical research community. Scientists said that many cancer research programmes would have to stop if liquid scintillation media and animal carcasses containing trace amounts of radioactive elements could no longer be disposed of in this way. Some medical schools claimed that storage space was so tight that research

could continue only for a few weeks if no action was taken.

Following this outcry, the contractor responsible for the Nevada and Washington sites announced that they would be reopened to receive laboratory wastes. However, the South Carolina site remains closed, and the two west-coast sites are being used by all major research laboratories which have not been able to obtain a licence for disposing of radioactive materials by other means.

Now NRC is proposing that liquid scintillation media and animal carcasses containing less than $\mu 0.05\text{Ci}$ of tritium or carbon-14 per gramme can be disposed of merely as toxic chemicals "without regard to their radioactivity".

The commission says that, since the potential dose to any individual exposed to these wastes would be less than 1 mrem a year, such a move would have a negligible effect on health aspects.

Using the same reasoning, it is also proposing that research laboratories and hospitals should be allowed to release up to 5 Ci of tritium and 1 Ci of carbon-14 a year into the sanitary sewage system, in addition to the present limit of 1 Ci per year for all radionuclides.

If these changes are approved, the commission estimates that research institutions and hospitals will save up to \$13 million a year. Disposal of the wastes by more conventional means would cost only \$3 million a year, compared with the \$16 million it now costs to package and ship the materials to the west coast sites.

The proposed change in the rules is intended to conserve waste burial capacity that is already in short supply at nuclear dumps. At present, between 200,000 and 400,000 gallons a year of liquid scintillation media — mainly toluene containing heavily diluted radioactive tracers — has to be stored at these sites, taking up 400,000 cubic feet of space, with animal carcasses taking up another 70,000 cubic feet.

Many university safety officers have welcomed the proposed changes in the rules. Others, however, are being more cautious, pointing out that the material will still have to be disposed of under strict federal, state and local regulations. Liquid scintillation media such as toluene are highly flammable and potentially carcinogenic — one of the reasons that South Carolina decided it no longer wanted to handle the wastes — and pose chemical and biological hazards which NRC says are a greater health problem than their radioactivity.

The result is that disposal will have to be carried out under strict new laws to deal with toxic wastes in general, such as the Resource Conservation and Recovery Act. The Environmental Protection Agency, however, has still to agree on how it intends to carry out the requirements of the act, and research laboratories are consequently uncertain of the new demands that they may have to meet.

David Dickson

Soviet cosmonautics

Cuban went up

All the most interesting aspects of Cuban science have been reflected in the Soviet–Cuban space mission, according to Wilfredo Torres Yribar, President of the Cuban Academy of Sciences. Half of the 20 experiments planned for the flight, he said, reflect the interests of the Cuban economy.

Although space research began in Cuba "just five years after the victory of the revolution", Yribar admitted that the initial contribution had been "rather modest". In the beginning, he said, space research had not even come under the Academy of Sciences, but had been supervised by the Ministry of Communications. However, for the past ten years, Cuba had been making extensive use of data from meteorological satellites. At first this was only for weather forecasting, but now, he said, the Meteorological Institute was working out programmes for monitoring industrial pollution and studying the influence of the earth's climate on the ripening of sugar cane.

Many of the experiments performed in orbit by Cuban cosmonaut Arnaldo Tamayo Mendez and his Soviet crew-mate Yuri Romanenko, appear to be simple extensions of the regular Salyut programme. As usual, there was an experiment to grow semiconductor materials using the Soviet-designed Splav furnace, while Cuba, as the non-Soviet participant, provided the experimental capsule — in this case, the target substance was epitaxial aluminium-doped gallium arsenide — and an appropriate code-name "Caribba".

The MKF-6 camera and the Bulgarian-made Spektr-15 apparatus (so named because it can record 15 radiation channels) have been standard equipment on several Salyut missions, although on this occasion they were used in the "Tropika" experiment (photography and field observations of a defined area of Cuba) and in "Antilla" — an examination of the dynamics of the physical, chemical and biological characteristics of agricultural land and part of the surrounding seas.

Medical experiments, too, are a regular part of such missions. In this case, they included a Cuban-designed "coordinograph", which determined the accuracy and time characteristics of the cosmonauts' coordination of right and left arm movements. Another new medical experiment, "Cortex", was described by the Cuban back-up cosmonaut Jose Lopez Falcon on Moscow radio: the cosmonauts, he said, are fitted with bonnets which include sensors and electrodes; light and sound signals are transmitted, and the data recorded.

The most specifically "Cuban" experiments, however, were "Sakhar" and

Forgotten martyrs

Perhaps the most interesting aspect of the Mendez–Romanenko flight is the light it throws on the early days of Soviet space research. Speaking last week on the twentieth anniversary of the Committees for the Defence of the Revolution, Fidel Castro paid tribute to the "scores of Soviet cosmonauts" who had paved the way for the Cuban flight. In particular, he recalled the "many who had died in their attempt to go into space or during their descent from space".

Those who died during descent are well known: Komarov in 1967 and the three crew-members of Soyuz 11 — Dobrovolskii, Volkov and Patsaev — in 1971. It is less clear who he meant by those who died attempting to go into space. Yuri Gagarin died in a plane crash in 1968, while still officially on the space team, and a back-up cosmonaut, Seregin, was killed with him. Two other trainee cosmonauts are believed to have perished in high-altitude training missions. The Soviet space planners have always been highly reticent about such accidents, and the rumours of the early 1960s often complete with cosmonauts' names and family details, seem for the most part to have had little or no foundation. Even Gagarin's historic flight was rumoured to have been the back-up mission to a disaster the previous day — a story which seems to have been generated by a premature TASS announcement — although no signs of any launch were picked up by the US monitoring stations in orbit. (Just conceivably, of course, a spacecraft could have blown up on the launch pad.)

Castro referred to having visited a "gallery of martyrs" at, presumably, Baikonur, where he observed that the loss of life had been "relatively high" before space flights were "made safer". Since no westerner — except possibly Charles de Gaulle — has ever visited this gallery, Castro's remarks have renewed speculation as to just whom it commemorates.

"Zona". These are designed to study the growth of sugar monocrystals in conditions of weightlessness — the first time an attempt has been made to crystallize an organic substance in orbit. Also of Cuban provenance is the "Support" experiment, which uses special Cuban-designed shoes that place a given load on the support areas of the feet. Returning cosmonauts, it seems, have a tendency to platypodia, and "Support" was intended to determine whether this change in the arch of the foot was a significant factor in post-flight posture disturbance. Although only Mendez wore the shoes on this occasion, they are to be worn by Soviet cosmonauts on future flights.

Vera Rich

CORRESPONDENCE

Non-proliferation

SIR — The widespread use of nuclear power plants has caused concern regarding the proliferation potential of commercial plutonium. In a recent article (*Nature* 28 February)¹, Lovins concluded that "with modest design sophistication, high-burn-up plutonium from power reactors can produce powerful and predictable nuclear explosions".

The disadvantage of using even-plutonium isotopes in a nuclear explosive stems from different physical factors, which might be underestimated if considered separately. An example of this is the effect on the critical mass (neutron multiplication and balance)^{2,4} or neutron generation time^{3,4} of using even-plutonium isotopes in a nuclear explosive. However, by considering (1) the neutron multiplication factor, (2) the neutron generation time, (3) the subcritical multiplication by approaching the criticality and (4) the neutron background due to spontaneous fission, we have found that the explosive yield of a nuclear bomb is decreased rapidly by the presence of significant amounts of even-plutonium isotopes. Our analysis showed that it would not be possible to exceed an explosive yield of 1 kton or 100 ton TNT in the presence of 15 or 25% even-plutonium isotopes, respectively, in the core of the critical assembly, even using high design sophistication.

However, even a low-yield fissile explosive can ignite a thermo-nuclear explosive. Also, the radioactive hazards of a low-yield nuclear explosive are substantial, as Lovins states¹. Hence the problems of commercial plutonium with regard to proliferation remain.

The nuclear power countries will not be able to prevent the non-nuclear power countries from going ahead with the commercial exploitation of nuclear energy. The only way in which non-proliferation can be attained will be through a political decision by the non-nuclear power countries.

SÜMER SAHİN

Laboratoire de génie atomique,
Swiss Federal Institute of Technology,
Lausanne, Switzerland

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2. Sahin, S. *Atomkernenergie* 27, 23-26 (1976).

3. Sahin, S. *An. Nucl. Energy* 5, 55-58 (1978).

4. Sahin, S. & Yalcin S. *Atomkernenergie/Kerntechnik* 36, 139-140 (1980).

5. Sahin, S. & Işigou J. *Nucl. Technol.* 50, 88-94 (1980).

Technical innocence

SIR — It seems likely that 37½ years in the Scientific Civil Service enables me to comment on your article (*Nature* 25 September, p.264).

Frustration and unease are not caused simply by the inability of administrators to comprehend the same "world-image" as we do; nor even is it just that they appear to take our advice or not according to lofty criteria that depend on the (? almost esoteric) concept of "policy" — which may sometimes not be within our own world-image, even though we may have been on more than one management

course. Apart from these things we are human beings. Therefore we resent the fact that an administrative or management mandarin is, even when of "equal button", always "equal and above", as expressed in terms of money and in advancement-potential. Scientists, said the late Walter Elliott, should be "always on tap, but never on top". This cynical dictum still "rules" — but it is not OK, and never will be. It is not necessary to try to turn us into "managers" or "administrators" in order to value us as equals, of whatever button. This is the true "parity".

Tolworth Laboratory,
MAFF, Surrey, UK

R. A. DAVIS

Read the small print

SIR — I wish to draw attention to a potentially dangerous trend in the presentation of scientific papers. In a paper published in this journal almost a year ago (Meltzoff, A.N. and Borton, R.W. *Nature* 282, 403-404, 1979) the widely publicized conclusion was that 1-month-old human neonates possess intermodal matching abilities. Although I feel that this conclusion, whether true or false, is derived by fallacious argument, my concern here is with a fault in the presentation of the paper; a fault which may account for the general acceptance of the paper's conclusion; and a fault encouraged in your "Guide to authors".

The authors of the paper use a modified version of a well tried, even "classical", experimental method in their investigation. Regrettably, they confine the description of their modifications to a densely printed figure legend, where it is embedded in the mass of relatively trivial detail intended for any who wish to repeat the experiment. More regrettably, the narrative of the paper carries what appears to be a complete account of an experimental method — the unmodified "classical" method — but it carries no hint whatever that this "classical" method has been modified by the manoeuvres which are mentioned only in the figure legend.

Thus, although all mention of certain heuristically significant experimental detail is omitted from the narrative of the paper, nothing suggests that the argument which is presented in that narrative, and which may impress the reader as being conclusive, fails to take account of all relevant factors.

When the description of experimental detail is fragmented as it is in this paper, there is a risk that fallacious conclusions may acquire an apparent but spurious validity, and a persuasive yet equivocal paper pass unchallenged into "the literature". There is, of course, no way of knowing how often the latter happens. Similarly, no amount of advice in a "guide to authors" can entirely eliminate its occurrence. But the practice of relegating experimental detail to figure or table legends is probably becoming more common; and the more common it is, the more dangerous: for readers learn to assume that authors *never*(!)

displace heuristically significant detail from the narrative of a paper without due notice.

Authors cannot be prevented from inadvertently misleading readers, even referees; but perhaps your advice to them should be reconsidered: "Experimental detail vital to the paper yet which would interrupt the narrative is best placed in the figure legends." — Is it?

DAVID BELASCO

Swaffham,
Norfolk, UK

Generating costs

SIR — Richard Marshall's letter (*Nature* 18 September, p.184) on the Central Electricity Generating Board's published cost forecasts for new nuclear and coal-fired stations, requires some comment. First, the load factor on any station in any year is a function of two parameters — the availability of the plant and the proportion of total electricity demand which it is economic to allocate to that particular plant. Nuclear stations for most of their life and new coal stations early in their life will normally be operated economically to the limit of their available capacity. In later years they will come a time for both types of plant when, because of the construction of still newer capacity, their load factor will fall below the maximum permitted by availability. Since coal-fired stations have a much higher operating cost than nuclear stations this process will start earlier for coal-fired stations, which will therefore achieve a somewhat lower lifetime load factor than nuclear stations.

The load factor projections are therefore not arbitrary; they derive from a careful estimate of expected economic loading on each plant in each year. The load factors so far achieved on the Hinkley B advanced gas-cooled reactor (AGR) station reflect the fact that this is the first commercial AGR station ever built, still in its early years of experience. Inevitably its availability to date reflects teething troubles. Our projections of future availability are made on the reasonable basis that in due course new plant yet to be built would benefit from this experience.

We would therefore not expect coal to achieve a 70 per cent load factor while nuclear achieved only 50 per cent, but even if this were to happen, the cost projections quoted by Mr Marshall are still incorrect. Accepting for the sake of argument 50 per cent and 70 per cent load factors for new nuclear coal-fired plant respectively, the adjusted generation costs would be 2.6 and 3.1 pence per kWh, thus still retaining the cost ranking as predicted. These figures differ from Mr Marshall's because only the fixed component of the generation cost varies with load factor when working in pence per kWh.

F. P. JENKIN

Planning Department,
Central Electricity Generating Board,
London EC1, UK

NEWS AND VIEWS

Nutrition and metabolism

from Mary Lindley

ON the top floor of the medical library at Johns Hopkins University a modest display reminds visitors that last year was the centenary of the birth of E. V. McCollum, discoverer of vitamins A and D. After becoming professor emeritus at Johns Hopkins in 1944, they are told, he spoke out against the fortification of foods, arguing that a well balanced diet was the key to good health. The visitors might reflect that since then, as fads and fashions have dictated nutritional trends, it has not always been clear what a well balanced diet might be, and the answer does not seem to be at hand. Delegates to a recent symposium* at Johns Hopkins on the effects of nutrition on metabolism may well have departed thinking that only the bravest would recommend a universally healthful diet, when food can have such apparently profound influences on the body.

One influence that has been fully appreciated only in the past year centers around the role of brown fat, which, as J. Himms-Hagen (University of Ottawa) pointed out, is now seen to have a fundamental role in the body's thermogenic response to food as well as to the cold. During the past twenty-five years brown fat (otherwise known as brown adipose tissue) has been seen as a possible site of thermogenesis, producing heat, especially in animals waking from hibernation and in the newborn. The homeostatic mechanism that keeps babies warm can also be demonstrated in rats exposed to a cold environment. They develop the capacity to increase their metabolic rate and thus maintain body temperature, in response to noradrenaline released by the sympathetic nervous system, whenever re-exposed to the cold. Muscle has also been suggested as the site of this non-shivering thermogenesis and only in the past few years has brown fat been firmly acknowledged as the tissue responsible.

The most recent stage in the story of brown fat has been the clarification of its role in generating heat in response to overfeeding. As M. J. Stock (St George's Hospital, London) explained, studies of human subjects had shown that some individuals generate heat in response to excess energy intake, but further investigation had been hampered by the lack of a

suitable animal model. Now Sclafani and Springer's cafeteria method has proved effective in inducing rats to supplement their diet of chow with delicacies such as bananas, biscuits and popcorn so that their voluntary energy intake increases by up to 80 per cent. Just like the human subjects, some of those rats do not become obese, but respond by generating heat. The site of this diet-induced thermogenesis turned out to be brown fat, pointing to a role for this tissue in energy balance as well as thermoregulation (Rothwell & Stock, *Nature*, 281, 31; 1979).

That idea has suggested a new way of looking at individuals who become obese in response to overeating. It is tempting to speculate that they have a defect in their brown fat and cannot dissipate the excess energy consumed. A hint that this may be correct is provided by the inability of obese animals to maintain their temperature in the cold, but, as W. P. T. James (Dunn Nutritional Research Laboratory, Cambridge) cautioned, a simple explanation of obesity is unlikely. Complications include factors such as thyroid hormone metabolism. E. Danforth (University of Vermont) explained that thyroid hormones regulate energy expenditure and are themselves regulated by diet; their metabolism is slow after a decrease in calorie consumption and accelerates after an increase.

The role of the sympathetic nervous system in the body's response to dietary stimulus is being investigated by L. Landsberg and J. B. Young (Harvard Medical School, Boston). They have measured noradrenaline turnover to show that sympathetic activity is suppressed in fasting rats and mice and stimulated when the animals are overfed by giving them access to sucrose in addition to their normal diet. The results indicate that the hypothalamus integrates those changes and that insulin may be an important link with the dietary stimulus. Landsberg suggested that such a system for controlling noradrenaline turnover, which is separate from the stimulatory effect of hypoglycaemia on the adrenal medulla, could be a mechanism to dissipate excess calories through thermogenesis and to conserve them when in short supply.

All this proved somewhat perplexing for the clinicians, who wanted to know what to

do with their obese patients if a reducing diet would depress the activity of both thyroid hormones and sympathetic nervous system. Danforth advised them to complement dietary treatments with a programme of exercise; diet alone will never be effective in controlling obesity, he said.

During the past ten years the brain has also proved to be subject to dietary influence in spite of the blood-brain barrier, which, J. H. Growdon (Massachusetts Institute of Technology, Boston) explained, was long thought to isolate it from the rest of the body. Variations in concentrations of choline and amino acids in the plasma can be correlated with fluctuations in the brain's content of neurotransmitters and their precursors. J. Fernstrom (Massachusetts Institute of Technology) explained that a diet containing largely carbohydrate and little protein stimulates the synthesis of serotonin, because its precursor tryptophan can enter the brain without competition from other amino acids, most of which are taken up by muscle. But when the diet is rich in proteins, competition from other amino acids prevents an increase in brain tryptophan, so that serotonin is not affected. The situation is different with tyrosine, the precursor of dopamine and noradrenaline. As C. Gibson (Massachusetts Institute of Technology) explained, tyrosine is more plentiful in food than tryptophan, and a diet rich in protein will supply sufficient of the precursor to enter the brain in spite of the competition and to influence the rate of synthesis of the two neurotransmitters.

A. J. Gelenberg (Massachusetts General Hospital, Boston) described how these three precursors are being used in clinical research as potential drugs to treat certain neurological disorders. Two examples are Alzheimer's disease, which involves recent memory loss and deficiency of choline acetyltransferase, and Parkinson's disease, a progressive motor disorder in which dopamine is deficient. Although it is not possible to assess the full therapeutic potential of neurotransmitter precursors, results so far have encouraged continuing studies.

As would be expected, malnutrition can have severe metabolic ramifications. The systemic immune response, for example, has often been shown to be subject to the vagaries of malnutrition, with T cells especially vulnerable. But N. F. Pierce

*The thirteenth Miles International Symposium, on Nutritional Factors: Modulating Effects on Metabolic Processes, was held at Johns Hopkins University, Baltimore on 18-20 June. It was organised by Dr R. F. Beers and the proceedings will be published by Raven Press.

Mary Lindley is an assistant editor of *Nature*.

(Johns Hopkins University) said that less is known about the response to invading microorganisms although they can have a devastating impact, particularly in the developing world where diarrhoea and respiratory infections are common afflictions of childhood. Using immunofluorescence to detect cells producing IgA, Pierce and his colleagues have examined the effect of protein malnutrition on the rat's mucosal immune response to cholera toxin. They found the response impaired in rats fed a diet containing 4 per cent protein compared with the usual 24 per cent.

F.B. Bang (Johns Hopkins University) reported that mice with a gene that normally makes them resistant to hepatitis will succumb to infection by that virus when maintained on a diet low in protein. In such cases malnutrition apparently affects the functioning of the macrophage system.

Malnutrition also influences infection by certain bacteria. E. Griffiths (National Institute for Medical Research, London) described how *Escherichia coli*, *Salmonella* and *Klebsiella* can sequester iron within their hosts. Those bacteria secrete enterochelin, which can remove iron from the binding proteins, transferrin in blood and lymph, and lactoferrin in mucosal secretions and milk. Once bound to enterochelin, the iron is taken up into the bacteria for their use. Of course the bacteria also have to contend with the host's immune system, which normally affords an effective defence. But children with the malnutrition syndrome kwashiorkor, which depletes transferrin, and also impairs the immune system, will be extra susceptible to infection from bacteria that can thrive due to the increased availability of iron, especially if iron therapy is started during refeeding.

W.E.M. Lands (University of Michigan, Ann Arbor) introduced a fresh aspect to the debate over polyunsaturated fatty acids, already controversial for their proclaimed ability to lower plasma cholesterol. Some of them can inhibit the conversion of arachidonic acid to prostaglandins and thromboxane, and their prevalence in the diet may influence the amount of those two substances produced in response to the appropriate stimuli. J. Dyerberg (Aalborg Hospital, Denmark) provided epidemiological and experimental evidence to support that suggestion. The Eskimos of Greenland, whose diet is rich in the polyunsaturated fatty acid eicosapentaenoic acid (EPA), a constituent of many marine oils, have a longer bleeding time and lower platelet aggregability compared with Caucasians. They also have a low incidence of myocardial infarction. In the experimental study, people given six grams of EPA per day for three weeks experienced a moderate decrease in platelet aggregation and prolongation of bleeding time. Dyerberg stressed, however, that the evidence so far does not justify recommendation of EPA

as an anti-thrombotic agent.

Those who are brave enough to attempt to formulate guidelines for healthy living based on the disparate influence of diet on metabolism must also remember the influence of genetics. For example, A.M. Altschul (Georgetown University, Washington DC) pointed out that the evidence linking salt consumption with hypertension indicates that there is likely to be a positive correlation only for people with a family history of hypertension.

B. Childs (Johns Hopkins University) issued a warning by recounting his cautionary variation on the nursery rhyme — Jack Spratt could eat no fat, his wife could eat no lean. Mr Spratt apparently avoided the consumption of fat because he had hypercholesterolaemia and his father had died of a heart attack. Mrs Spratt, on

the other hand, became dizzy and sick whenever she ate food rich in protein. Due to a mutant gene on one of her X chromosomes she had a partial deficiency of ornithine transcarbamylase, preventing the normal production of urea, and leading to the accumulation of toxic ammonia.

For Mrs Spratt and those with other defects of the urea cycle some hope seems to be offered by dietary manipulation facilitating the removal of waste nitrogen in some form other than urea; for example, supplementation with arginine increases the production of arginosuccinate, which is excreted by the kidneys. Although such examples represent the extreme of sensitivity, individual responses to dietary variation are likely to span a wide range, making the ideal diet impossible to formulate. □

Scanned image microscopy

from I. R. Smith and D. A. Sinclair

A recent conference* showed that new types of scanning microscope which promise unique imaging capabilities are rapidly being developed. As well as improved optical microscopes, X rays and acoustic waves are being used for high resolution scanned imaging and energy conversion techniques are being employed in the design of photo-acoustic and photothermal microscopes.

In a scanning microscope a tightly-focused beam is scanned over the specimen and the transmitted or reflected energy is made to modulate the brightness of a scan-synchronized display to form an image. The resolution of the system is dependent upon the width of the beam (in general, comparable with the wavelength of the radiation employed), whilst the image contrast depends upon the interaction of the radiation with the specimen. The development of new types of microscope is possible because the constraints upon the focusing elements of a scanning microscope are relaxed when compared with an imaging one. In particular, wide field performance is not required.

Scanned optical microscopes (SOM) are a factor of 1.4 times better in resolution than their imaging counterparts because of the use of a confocal lens arrangement in which the 'condenser' lens itself forms a diffraction limited spot upon the specimen (G. J. Brakenhoff, University of Amsterdam). Resolution may be further increased by the use of shorter wavelength ultra-violet radiation. Still higher

resolution may be obtained in electron microscopes but these are unsuitable for imaging living cells. Scanning microscopes allow a great diversity of operating modes. One, demonstrated by T. Wilson (Oxford University), uses the SOM to probe semiconductor devices. The focused laser source of the SOM generates hole/electron pairs within the semiconductor, and these can be measured by monitoring the current in a p-n junction. The image shows how much current was generated over the device, and regions of low generation indicate the presence of electrically active defects, such as impurities, dislocations and grain boundaries, which can contribute to device failure.

X-ray microscopy has potentially very high resolution. At a wavelength of 3nm there is a large difference in the X-ray absorptivity of water and protein so it is possible that images of living cells can be obtained with both high resolution and contrast. Only synchrotron sources are currently bright enough for microscopy so widespread use must await the development of a compact, coherent X-ray source.

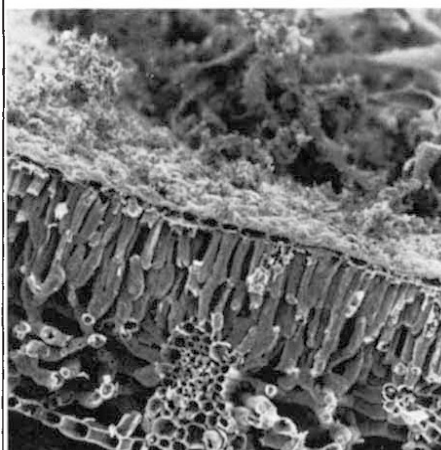
Above the 'soft' ultra-violet region, conventional refracting optical components become highly inefficient. Three solutions to this problem are being tried. In the first an unfocused X-ray beam passes through a very small (μm) aperture which is scanned over the object (H. Rarback, University of New York). The

*An international symposium on 'Scanned Image Microscopy' was held by the Royal Society, London, 22-24 September, 1980. The conference proceedings, edited by E. A. Ash will be published by Academic Press, November, 1980.

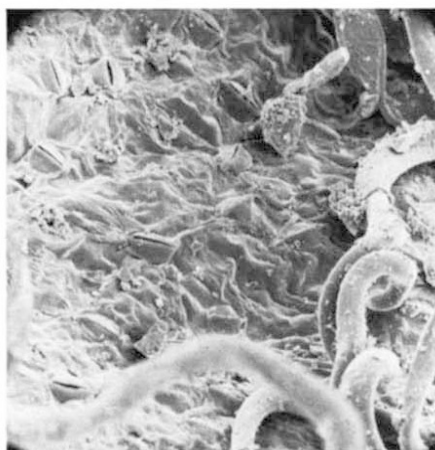
I. R. Smith and D. A. Sinclair are Research Assistants in the Department of Electronic and Electrical Engineering, University College London.

Ash from Mt St Helens

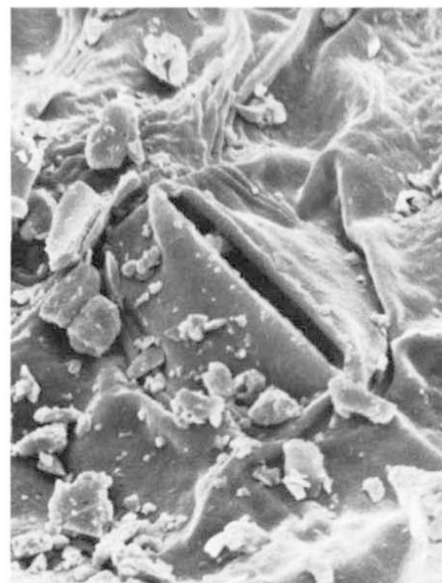
from Robert A. Kennedy



Cross section of apple leaf with ash on the upper epidermis.



Lower apple leaf surface showing ash among the trichomes and stomata.



Ash inside a stoma on the lower epidermis of an apple leaf.

AFTER the May 18th explosion of Mount Saint Helens ash fell on nearly seven million hectares of land in the State of Washington and in some places reached depths of 75cm. The pictures of plant leaves shown above were taken one and a half months after the explosion at Pullman, Washington, more than 250 miles from the volcano. Although over

three inches of rain had fallen since the eruption large amounts of ash particles remain on the leaf surfaces, coating both the upper and lower epidermis and even finding their way into stomata.

The direct effect of the ash is to reduce light penetration; a one mm thick coating on the leaf absorbs 90 per cent of the light and virtually stops photosynthesis. More

indirect and longer term effects may come from the presence in the ash (in concentrations of up to 10ppm) of the toxic element fluoride.

Robert A. Kennedy is associate professor in the Department of Horticulture and Landscape Architecture, Washington State University.

aperture defines the beam width, and hence the resolution. The second approach is the use of a Fresnel zone plate for focusing the radiation (G. Schmahl, University of Göttingen; N. M. Ceglio, Livermore Laboratories), a technique which has resulted in 0.15 μm resolution. The third method uses a focusing mirror with multi-layer coatings to increase reflectivity (E. Spiller, IBM New York; R. P. Haelbich, University of Hamburg) and seems likely to yield the highest resolution.

The development of scanning acoustic microscopy (SAM) has two main objectives; the attainment of at least sub-optical resolution and the determination of the object's elastic constants. A sub-optical resolution microscope implies the use of a low-velocity, low-loss acoustic coupling liquid. Lemons (1974) has defined a coefficient of merit, M , which normalises the resolution attainable with a particular liquid to that with water — the liquid conventionally used in the SAM. Cryogenic liquids, for example, Argon at 87K for which $M=3.7$ and Helium at 1.95K and 0.4K for which $M=3.7$ and 23 respectively offer potential improvements in SAM resolution. J. Heiserman (Stanford University) described a cryogenic reflection SAM designed to use these liquids and which currently works with liquid Helium down to 1.95K where the wavelength is 360 nm. The banded structure of human chromosomes has been clearly imaged with this system.

High pressure gases are, as pointed out by H. K. Wickramasinghe (University College, London), an attractive alternative to cryogenics since $M=4$ and 5 for Xenon and Argon at 40 and 250 atmospheres respectively. This suggests that resolutions just less than 100nm can be achieved. Room temperature operation is an additional advantage that will allow simple sample preparation techniques and the imaging of living cells.

To measure an object's elastic constants C. F. Quate (Stanford University) described a technique in which the output voltage, V , of a reflection SAM is recorded as a function of defocusing distance, z , at a single image point. The $V(z)$ curve obtained is a characteristic function of the elastic properties of the object surface. Both the density and the longitudinal velocity of the sample may, under certain conditions, be computed in this way.

The SAM has been used to image a wide range of objects. Coal may be characterised, as suggested by C. F. Quate, by its acoustic reflectivity. This technique should offer an increase in sensitivity of forty times over the comparable optical technique. He also showed using $V(z)$ techniques that the SAM is eminently suitable for detecting delaminations in thin films such as chrome on glass. The large impedance mismatch between the surface of a metallic object and the water coupling the lens to the object may be overcome by

replacing the water with a liquid metal such as mercury or gallium. This and the use of shear waves in addition to the liquid metal coupling can provide improved resolution due to the decreased wavelength and reduced spherical aberration in the object (J. Attal, Université des Sciences et Techniques du Languedoc).

Interest in the photoacoustic effect, first demonstrated by Bell in the 1880's, has increased dramatically in the last decade with the development of several photoacoustic imaging systems capable of microscopic resolution. In what has become conventional photoacoustic microscopy (PAM) a modulated light beam is scanned across a solid surface. A microphone is used to detect the pressure waves generated in the gas above the surface by virtue of the periodic heating at the sample surface. Since a lock-in amplifier is normally used in the detection system both the amplitude and phase of the photoacoustic signal can be mapped out across the sample. A. Rosencwaig (University of California) and G. Busse (ZWE Physik) pointed out the components of the signal detected at different phases relate to information from different depths within the sample. The system resolution is determined partly by the optical spot size and partly by the thermal wavelength in the solid. This latter quantity is inversely proportional to the square root of the beam modulation frequency and affects the depth of penetration of the thermal wave in

the solid.

G. Cargill (Watson Research Centre, IBM) and Rosenwaig showed that the beam in a SEM may also be used to generate a thermal wave in a sample. A piezoelectric detector bonded to the back surface of the sample collects the acoustic wave generated by the thermal expansion in the solid. In this technique of electroacoustic microscopy much smaller spot sizes can be used to illuminate the sample and the resolution is then only limited by the thermal wavelength. Submicron resolution should be possible using 1-10 MHz modulation frequencies. Rosenwaig demonstrated the detection of a 2 micron crack beneath a solder pad in a ceramic substrate. By selection of the appropriate phase of the electroacoustic signal subsurface imaging is possible. He presented images showing phosphorous

dopant regions in a Si substrate beneath a SiO₂ layer.

A technique related to PAM again uses a modulated light beam to illuminate the sample surface. An infrared detector is, however, used to directly, but remotely, monitor the sample surface temperature. The detector output is a function of both the optical and thermal properties of the sample. P. Nordal (Laser and Applied Optics Laboratories, Blindern) showed that this technique of photoinfrared microscopy was sufficiently sensitive to detect a single monolayer of aspartic acid in a chromatogram.

In conclusion, scanning microscopes offer higher resolution and a natural imaging environment for living cell cultures, whilst in the field of non-destructive evaluation, give information inaccessible to conventional techniques. □

Gluon fusion

from V. Barger

THE production of particles made of charm or b-flavor quarks has been under intensive study for several years. Of special theoretical interest in the production of these quark flavors is the large mass scales involved, which permit perturbative calculations of strong forces using the theory of quantum chromodynamics (QCD). Recent data from experiments with muon beams at CERN¹ and Fermilab² give evidence for copious production of charm and anticharm, which can be compared with QCD expectations. The successful description of these data was a major topic³ at the XXth International Conference on High Energy Physics held at the University of Wisconsin-Madison in July.

A muon beam (μ^+ or μ^-) interacts with a nucleon target via the electromagnetic interaction through the exchange of a virtual photon. In the scattering of the

virtual photon with a gluon constituent (g) of the nucleon, a charm-anticharm quark pair ($c\bar{c}$) can be created (Fig. 1). These quarks materialize with probability unity as charmed hadrons through the confinement force. The subsequent weak decays of the charmed particles can lead to additional muons (Fig. 2), which provide a clear experimental signature for charm events. On the average, the decays of a charm particle yield a muon about 10 per cent of the time, with a μ^+ originating from charm decay and a μ^- from anticharm decay. Counting the primary muon from the beam scattering, charm pair production results in dimuon and trimuon events. The muon emitted in charm decays is accompanied by a neutrino (ν), whose energy and momentum are unobserved. Thus multimMuon events from $c\bar{c}$ production are characterized by missing energy.

Unlike the muon signals from charm

decays, multimMuon sources from other electromagnetic processes mediated by two virtual photons have zero or little missing energy. Fortunately, contributions from these non-charm sources can be reliably estimated.⁴ By appropriate experimental cuts the charm signal can be isolated.

The theoretical calculation of photon-gluon fusion⁵ (Fig. 1) depends on the strength of the gluon to $c\bar{c}$ strong interaction coupling, which is fixed by QCD analyses of other reactions. Another necessary ingredient is the probability density for finding a gluon carrying some fraction of the nucleon momentum. From inelastic electron scattering, it is known that gluons carry about half of the nucleon's total momentum. Using conventional theoretical prejudices about the shape of the gluon probability distribution,⁶ the predictions of the photon-gluon fusion model are found to be in excellent agreement with the multimMuon data.

The fusion approach also applied to the production of bound $c\bar{c}$ states, such as the ψ -particle. It is assumed⁷ that ψ -production is proportional to the density of $c\bar{c}$ states produced near the ψ -mass. The model is in good agreement^{8,9} with "elastic" ψ -production events^{1,2} that have little accompanying hadronic energy. For inelastic ψ -production additional fusion diagrams enter, with an extra quark or gluon carrying away energy.¹⁰

In hadron-hadron collisions the creation of $c\bar{c}$ or $b\bar{b}$ pairs occurs via gluon-gluon fusion and via the fusion of light quarks q with their antiquarks $\bar{q}$ (Fig. 3). Data on the hadroproduction of the ψ and Υ particles allow the extraction of gluon distributions for pions, kaons, and nucleons. A consistent fusion description of the hadroproduction of heavy quark bound states emerges,⁹ with the same gluon distribution for the nucleon as determined from virtual photoproduction.

These successes of the fusion model are somewhat clouded by the present situation concerning the hadroproduction of unbound charm, where the predicted rates are too small. One possibility is that yet to be calculated higher order QCD diagrams are important in hadron-hadron collisions. □

Fig. 1

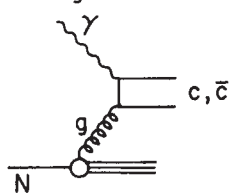


Fig. 2

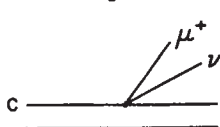
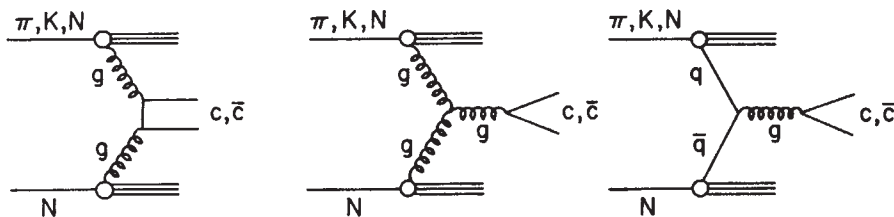


Fig. 3



V. Barger is Professor of Physics at the University of Wisconsin-Madison.

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On the Lost Art of Seeing where Things are Going

from Simon Heywood

No one disagrees that one of the most important perceptual tasks to confront a sophisticated and mobile organism is the capacity to detect and discriminate movement, whether of itself or of other organisms or objects. It therefore seems obvious to devote research to the processes underlying the perception of movement, whether in terms of the psychological or phenomenological characteristics of such processes or their putative realisation in neural machinery.

A recent NATO-sponsored symposium* called together some eighty workers in the area to review and to extend our knowledge of these processes. However, it must be admitted that simple as the problem of movement perception may sound, four days of conference served to illustrate that there remains considerable theoretical disagreement over what the proper object of study actually is. The explosion of "facts" unearthed by more or less ingenious physiological, psychophysical and psychological experiments has all too clearly not been paralleled by a development of theory to establish the meaning of such facts.

While it is obvious that we can and do perceive movement (just as we can and do perceive form or colour) it cannot be assumed that, therefore, the logic of investigating its physiological substrates is the same. Microelectrode studies reveal that a significant majority of visual cells in the cortex or the midbrain of animals respond to the movement of stimuli through their receptive fields; studies of great elegance classify the response types of these neurons in terms of stimulus velocity, or of their sensitivity to stimulus position change or of the position of their receptive fields and so on. But a moment's thought suggests that the relevance of these studies to the perception of movement is entirely unclear. In the great majority of visual environments, the predominating mass of visual objects and textures are actually stationary; a small subset moves. However, for any organism in motion (especially one with highly mobile eyes, like ourselves) the retinal images are constantly moving. The inescapable conclusion is that in order to interpret visually what is actually moving in the world we must first be able to establish the stability of the visible frame-of-reference within which and with reference to which that movement occurs. Single unit studies have (with a few notable exceptions) indicated only that cells respond to retinal stimulus motion, and

hence fail to tell us whether such cells subservise the perception of movement or that of stability.

The simplest (and the oldest) solution is to argue that we establish stability by combining the retinal movement information with information about eye and head (and possibly body) movement. Thus perceived stability is achieved when the movement on the retina is matched and congruent with movement produced by the organism's oculomotor and neck musculature. In this case, the movement sensitive visual cells could at best only be understood in the context of other non-visual information, about whose distribution in the brain we are at present almost entirely ignorant.

Unfortunately two kinds of experimental results cast doubt on even this as a basis for the perceived stability (and hence perceived motion) of objects in the world. First, any such corollary signal from the eye and head moving systems to the perceptual apparatus appears to be far too 'sloppy' to achieve the nicety of stabilisation that we perceive. Second, recent work, reported at the symposium, shows that high-level cognitive processes may be more important in assigning stability or movement than the exact relations between retinal image shift and eye/head movement. What is important, and dominant, in perception is the visual *relationship* between objects and frames-or-reference; it appears that the perception of movement in natural environments can be understood primarily as an object-relative phenomenon. This, of course, makes the interpretation of electrophysiological data even more problematic.

However, consensus does appear to be emerging on broad lines of how the visual

perception of movement and stability may have to be structured; a number of speakers from quite different research backgrounds were echoing dichotomies first proposed in 1968 by Schneider, Trevarthen, Ingle and Held. There appear to be many ways of expressing these dichotomies and it is not yet clear how precisely they relate to each other and at what levels of description they operate, but three that were frequently mentioned were a) a distinction between a "movingness" (velocity-based) and a "displacement" (amplitude-based, hence perhaps related to position) system, using Claude Bonnet's terminology; b) a distinction between "central" and "peripheral" vision (which is not necessarily to be taken as an anatomical distinction, though there is clearly a spatial differentiation between them), and c) a distinction between two modes of oculomotor activity, one designed to hold part of the retinal image still on the retina and assumed to be primarily driven by retinal image velocity, and the other designed to shift the retina fast underneath the retinal image and assumed to use primarily positional information. Such dichotomies are clearly interrelated and related to the dichotomies used elsewhere between sustained and transient channels, or ambient and focal vision, or x and y cell systems, or saccades and pursuit eye movement. Perhaps if we could devote the next symposium not to the presentation of many new facts, but to the elucidation of these concepts and their functional relationships, we should move closer to understanding not only our perception of movement but how such perception is related to the structure and control of our own activity. □

Optical discs for information storage

from A. E. Bell

THE high density optical disc may provide a new way of meeting the need for low cost information storage. A 12" diameter optical disc can store as much as 10^{11} data bits, and recording rates up to 50 Mbits s^{-1} for a single channel have been demonstrated. By comparison to magnetic disc recording, optical recording increases the data packing density by about two orders of magnitude, producing a much lower cost per bit stored.

The key component of the optical system is the recording medium and several possibilities, including an organic dye-in-polymer¹, generally satisfy the requirements of recording sensitivity and

playback signal quality. Current research is now concentrated on improving the permanence of the information once stored as this is a key factor in the final selection of a suitable recording medium.

A typical high density optical recording system is shown in Fig. 1. The recording light source is a gas laser which emits a continuous, collimated and linearly polarised beam with a very well defined wavelength. The beam passes through a modulator to which the input data signal is applied and then via some optics and a polarising beam splitter/quarter-wave plate combination into the entrance pupil of the focusing lens. The lens focuses the intensity modulated beam to a spot on the recording medium, coated onto the surface

*Symposium on the Study of Motion Perception, Veldhoven, The Netherlands, 24-29 August 1980.

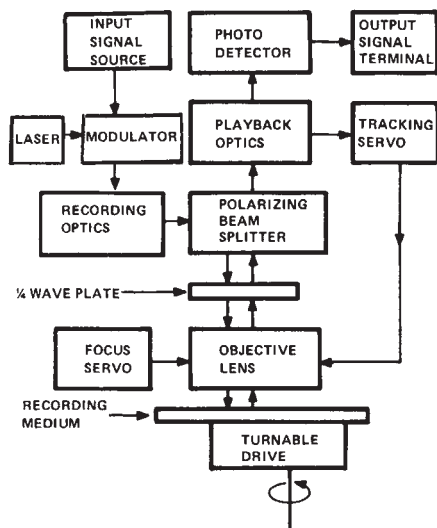


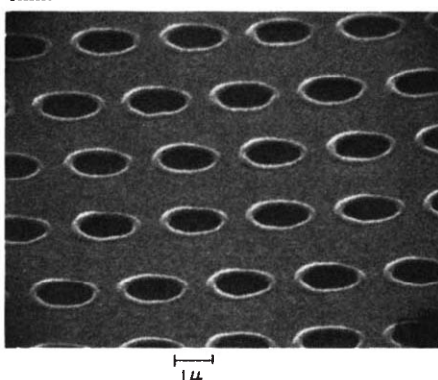
Fig 1 An optical disc recording system.

of the rotating disc substrate. Where the incident intensity is above threshold the recording medium is melted or vaporised so that the a record is made as a series of micron-sized pits along the track (Fig. 2). These change the local reflectivity (or transmissivity) of the medium permitting optical playback of the recorded data.

To retrieve the data, the laser intensity is reduced below the recording threshold and a low intensity read spot is focused onto the information track. The intensity of the reflected beam is modulated by the pits and the reflected beam is collected by the focus lens and directed back towards the polarising beam splitter. As a result of the double passage of this read beam through the quarter-wave plate, the polarising beam splitter separates the read beam and directs it via the playback optics chain to a photodetector. The electrical output of the photodetector thus represents the original input data signal.

Three basic servo control are required by the optical recording system. The focus servo continuously adjusts the position of the focus (objective) lens to compensate for the unevenness of the disc substrate, and maintains the focused recording or readout spot in the plane of the recording medium. The tracking servo ensures that during playback the readout spot is

Fig 2 Scanning electron microscope image of melted pits recorded in a 30 nm thick tellurium film.



centered on the information track of interest (the separation of tracks can be as low as two microns). The turntable servo controls the speed of rotation to preserve timebase information.

The simplest²⁻⁴ recording medium is a disc substrate coated with a single vacuum deposited layer of metal (~ 30 -50 nm thick). As the recording mechanism consists of melting the coating to form a submicron diameter pit surrounded by a rim of resolidified material (Fig. 2), the quality of the playback data signal is largely determined by the geometrical regularity of the pit shape. The process by which the melted pit is formed is currently being investigated and while no complete detailed model exists it is clear that surface tension, viscosity and the presence of nucleation centres on the substrate can all play an important role.

The laser power required to melt the pit is determined by the thermal characteristics of the metal layer. The element, tellurium, which has unusually low thermal diffusivity and a melting point of 452°C, has been widely investigated.⁴ Since the metal layer is so thin, even during the rapid recording pulse (a data rate of 10 Mbit s⁻¹ corresponds to a 50 ns recording pulse) a significant fraction of the heat generated within the metal layer can be conducted into the substrate. To avoid this the thermal diffusivity of the substrate material should also be as low as possible. Low cost plastic substrates such as poly-(methyl methacrylate) or poly-(vinyl chloride) satisfy this requirement.

Of equal importance in determining the sensitivity of a recording medium is the fraction of the incident beam which is absorbed. As in single layer structures about 40 per cent of the beam may be reflected, antireflective coating configurations have been proposed⁵ and investigated.^{6,10} The bilayer structure, Fig. 3a is most suited to moderately absorptive materials such as organic dyes, or organic dyes in polymer mixtures. A highly reflective aluminium layer is first vacuum-deposited onto the substrate. The recording medium itself is then deposited (by spin-coating or vacuum deposition) to a thickness of approximately one quarter-wavelength of the recording beam in the recording medium (that is, $\sim \lambda/4n$). This thickness results⁵ in a very low reflectivity with up to 90 per cent of the incident beam being absorbed in the recording medium. When the pit is formed, the interference condition is disrupted and the pit exhibits a very high reflectivity, as a result of the exposed, but unperturbed, aluminium layer. The bilayer approach thus gives a high sensitivity and a high playback signal contrast between the reflective pits and the antireflective lands in between.

Another approach which permits low reflectivities to be obtained even with strongly absorbing metallic recording media is the trilayer structure, Fig. 3b. The aluminium reflector layer is first coated

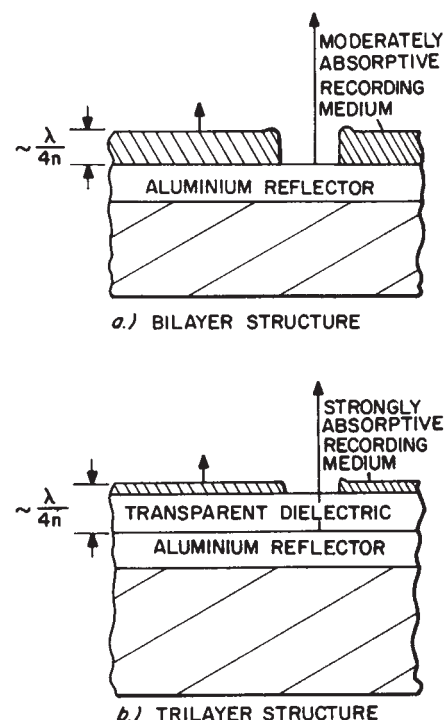


Fig 3 Antireflection structures for optical recording media.

with a transparent dielectric (such as silicon dioxide) and then a very thin layer (5-10 nm) of the highly absorbing recording medium. By manipulating the thicknesses of the dielectric and absorbing layers,⁵ the phase and amplitude relationships required to produce destructive interference and thus low reflectivity, can be separately controlled. In a low reflectivity design up to 95 per cent of the incident beam is absorbed by the 5-10 nm thick layer of recording medium. Melting of the thin layer of recording medium disrupts the interference condition and results in a highly reflective pit. The reduced thickness of the metal absorber in the trilayer structure as compared to the single layer structure leads to improved signal-to-noise ratio (more uniform pit rims). Furthermore, the antireflection properties of the trilayer structure can be 'tuned' to a variety of recording wavelengths simply by adjusting the thicknesses of the dielectric and absorbing layers.

Some problems are common to any high density data disc design; these include the substrate preparation and the encapsulation of the recording medium to protect it from dust particles. Clearly, since the density of data storage is very high ($\sim 10^8$ bits cm⁻²) even microscopic defects in the substrate disc can result in significant loss of data. Highly polished glass surfaces approach the level of perfection required, but they are both expensive and bulky. Low cost molded plastic substrates have a high level of surface imperfections and require surface improvement before use. The simplest procedure is to spin-coat the disc substrate with a thin layer ($<20 \mu$) of liquid polymer resin which is then cured either by heat treatment or UV irradiation.

The surface defects of the original substrate surface are filled in and the cured surface of the polymer layer can match or even exceed the surface quality of specially polished glass.

As dust particles falling onto the recording medium would rapidly introduce errors, the direct encapsulation of the recording medium with a relatively thick (180 μ) layer of transparent polymer may be a valuable final step in the disc fabrication procedure. Dust particles will be on the outside surface of the encapsulation layer and out of focus.

High density optical recording may have

very wide applications including the storage of computer, satellite, financial, X-ray and government statistics data, as well as video pictures. At the moment, the key problem is the permanence of the optical data as the minimum useful lifetime for most large volume applications is about 10 years. Of particular significance is the continuing development of relatively high power solid state diode lasers⁹ and compatible media^{10,11} since by comparison to the gas lasers these devices offer compactness, low power consumption, direct modulation capability and potentially increased reliability. $\square$

synapse elimination is the postnatal segregation of thalamic projections to the visual cortex of monkeys and cats⁷. At birth, axon terminals activated by one or the other eye are distributed throughout layer IV of the primary visual cortex, and may contact the same cortical neurons. During the subsequent weeks, however, the projections from the two eyes become separated so that cortical neurons in this layer are ultimately driven by either the left or the right eye. The axon terminals carrying information from the two eyes actually become segregated into domains that have the appearance of zebra stripes when mapped on the cortical surface. While the mechanism and purpose of this segregation in the brain remain a mystery, the underlying rearrangement of connections is in many ways similar to the winnowing out of some initial synaptic contacts in the periphery. In several parts of the nervous system, then, there is a gradual tuning of synaptic connections. These adjustments are competitive in the sense that the fate of particular terminals is not predetermined, but depends on an interaction with the target and with fellow axons.

The idea of a competitive balance of synaptic connections is by no means the exclusive province of developmental neurobiologists. It is well known that mature axons sprout and make additional synapses if their target is partly deprived of innervation, and, conversely, that adult axons can reduce the number of synapses they make on target cells under a variety of conditions⁸. These phenomena suggest that the number of synapses present in maturity continues to be regulated by interactions at the level of the target, much as competition between neurons for survival is regulated at an earlier stage of life.

What, then, is the relevant target property? A clue to the regulatory mechanism of both cell death and the ultimate equilibrium of maturing synaptic

Neuronal competition

from Dale Purves

ALTHOUGH the idea of competition between nerve cells during development is hardly new, it is enjoying a distinct renaissance. There are several good reasons for this flurry of interest.

Early evidence for a competitive interaction between neurons came from the work of Hamburger and Levi-Montalcini¹. They showed that about half of the nerve cells initially generated in some parts of the vertebrate embryo die during later development, and that the amount of this cell death is modulated by the neuronal target. Thus the vast majority of neurons in a population degenerate if their intended target (the limb, for example) is removed early in development and, conversely, the incidence of cell death is reduced if an additional target is provided (by implanting a supernumerary limb²). Taken together, these findings show that the death of some neurons is not a predestined affair, but depends on the ability of individual nerve cells to respond to some property of the limb. The importance of the target in regulating cell death by a mechanism that is, in a broad sense, competitive has been widely confirmed, most recently in a detailed study of the dependence of ciliary ganglion cells on the iris³.

As with any idea that threatens to become a dogma, there has been some dissent, most recently expressed by Lamb⁴. He reported that when both right and left hindlimb motor neurons of the frog are made to project to a single limb at an early

stage of development, the total number of surviving motor neurons in the relevant part of the spinal cord is greater than the number of neurons that normally survive to innervate that limb. He concludes that the "limb does not limit the number of survivors and therefore the hypothesis (of peripheral competition) is refuted . . .". Many neurobiologists, however, will take this result as an argument against a particular (one-to-one) conception of the neuron's dependence on its target, rather than an argument against competition as the basis of nerve cell death in normal development. Lamb's result suggests that the number of surviving neurons is not related to the amount of available target in a simple linear manner. A simple relation is not expected if neurons regulate the target property that they seek; a plausible notion since many properties of muscle fibers are regulated by the innervation they receive.

A central reason for renewed interest in the idea that neurons compete for some aspect of their target is that it seems likely that competition also regulates the number and arrangement of synaptic connections in the nervous system. Several phenomena in development and maturity have led to this view. For example, both muscle fibers and autonomic ganglion cells are innervated by more axons at birth than they are a few weeks later, indicating an extensive rearrangement of connections in early postnatal life⁵. In the central nervous system, a probable counterpart to the early elimination of some synapses in the periphery is a reduction in the number of climbing fibers innervating each cerebellar Purkinje cell in neonatal rats⁶. An especially intriguing example of central

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Dale Purves is Professor of Physiology and Biophysics, Washington University School of Medicine.

connections is the action of the protein called nerve growth factor (NGF). In the early 1950s, Levi-Montalcini, Hamburger, and their colleagues⁹ extracted from a mouse tumour an agent that stimulates the growth of two types of mammalian neurons (sympathetic and dorsal root ganglion cells). Long considered a curiosity rather than a molecule of fundamental significance, NGF's biological importance has now been firmly established¹⁰. In brief, neonatal animals immunologically deprived of this agent grow to maturity with dramatically stunted sympathetic ganglion cells¹¹ while, on the other hand, neurons in ganglia surgically isolated from their target can be temporarily rescued from death by exogenous NGF¹². These trophic effects are associated with NGF binding to specific receptors on the peripheral processes of sensitive neurons, and subsequent internalization and retrograde axonal transport¹⁰. The structure of the molecule is now known, and several groups have begun to unravel its mode of action¹³. Thus, for some neurons, there is con-

siderable evidence that the object of competition in the struggle for survival is NGF produced at or near the ganglionic target. The weakest link in this argument for the function of NGF has been the inability (for technical reasons) to demonstrate in an unequivocal manner production of the protein by the usual targets of NGF-sensitive neurons. This part of the story has now been considerably strengthened by a report that at least one sympathetic target (the iris) does indeed produce NGF under appropriate conditions¹⁴. Since it is clear that NGF's influence is limited to a small proportion of neuronal types, a major effort is underway to discover agents presumed to operate in an analogous manner in other parts of the nervous system¹⁵.

A further aspect of the effects of NGF suggests that a common mechanism may underlie a neuron's struggle for survival and the later competition between axons during synapse formation. Campenot¹⁶ has shown that terminal processes of sympathetic neurons are influenced by local

concentrations of NGF *in vitro* in a way that seems closely related to the proliferation and regression of synaptic connections observed *in vivo*. In Campenot's experiment a special culture chamber was used that allowed cell bodies and their processes to be exposed to media of different composition. When the neuronal processes were bathed in medium containing NGF they remained healthy; terminal processes regressed, however, when NGF was available to cell bodies, but absent in the vicinity of the processes. An attractive inference, therefore, is that competition of nerve terminals for trophic factors is also involved in the formation and maintenance of synaptic connections.

Neuronal competition is thus emerging as the common denominator of important processes (neuron death, neonatal synapse elimination both in the periphery and centrally, synapse proliferation and regression in maturity) that previously seemed unrelated. As such, it is likely to be one of the dominant themes in neurobiology for some time to come. □

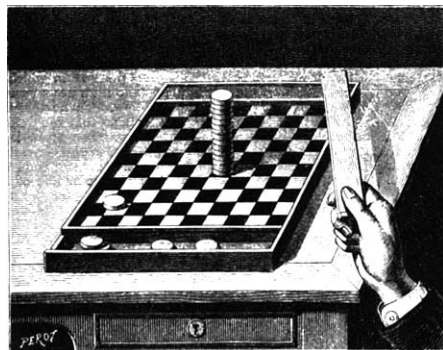


100 years ago

The monster python which is kept alive in the Antwerp museum having had inflammation of the jaw, a Belgian doctor volunteered to enter its cage in order to cure it; but the brute attempted to suffocate the poor doctor, who was glad to escape with his life.

PHYSICS WITHOUT APPARATUS

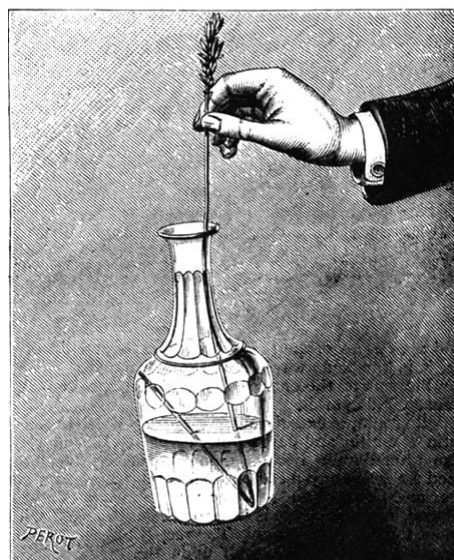
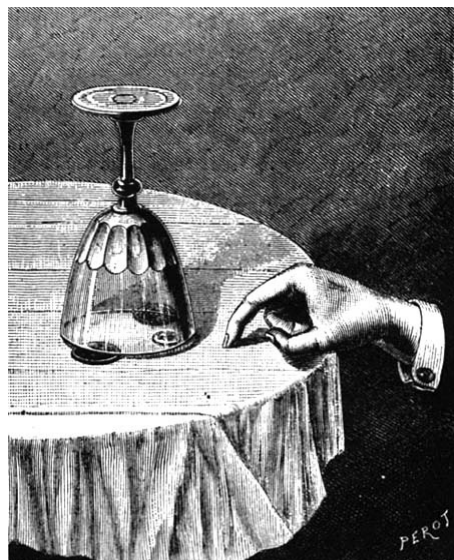
Inertia may be demonstrated by the following trick: a number of the round wooden "men" used in playing the game of draughts are piled up in a column one upon another. If the lowest one of the pile is dextrously hit with the edge of a paper-knife or other suitable article it may be knocked away from under the others without overthrowing the others. The figure shows how the experiment is arranged, the narrow slip of wood which serves as the lid of the box being here used as the weapon. Beginners in science must not mistake the meaning of the term *inertia* as applied to matter. Matter is



not in itself lazy or inert. But it possesses the property of *mass*, and to set mass in motion requires the expenditure of *energy*. If we skilfully spend the energy of the rapid blow upon the one draughtsman, it is knocked away before there is time for any considerable part of the energy to be imparted to the others that are piled upon it.

Another simple experiment, depending partly upon the inertia of matter and partly upon elasticity, is often shown as an after-dinner trick. Upon a linen tablecloth is placed a threepenny-piece between two pennies or other larger and thicker coins. Over this an empty wine-glass is placed, and the puzzle is how to get out the smaller coin without touching the glass. The very simple operation of scratching with the finger-nail upon the cloth, as shown, suffices to accomplish the trick, for the little coin is seen to advance gently towards the finger until it has moved completely away from under the glass. The fibres of the linen cloth are elastic; when you scratch with your finger-nail they are drawn gently forward until the force of their elasticity becomes too great and they fly back, to be once more drawn forward, again to slip back, and so on. While the fibres are drawn forward slowly, they drag the coin with them to a minute distance. But when the slip occurs and they fly backward, they do so very rapidly, and slip back under the coin before there is time for the energy of their movement to be imparted to the coin to set it in motion. So the coin is gradually carried forward over the surface of the cloth.

We will next give a simple experiment which illustrates the principle that a substance which is very weak in one direction may be very strong in another. It is possible to lift a decanter full of water by means of a single straw. To do this the straw must be bent as shown, so that the weight comes longitudinally upon the straw. The straw is a very weak thing if it has to resist a force applied laterally. Lay a single straw horizontally, so that the two ends are supported, and then hang weights on to the middle of it: a very few ounces will break it across. But let the weights be fixed to one end of the straw, and the straw itself be hung downwards so that the pull is exerted along it, and it will support one or two pounds at least.



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ARTICLES

A low velocity zone beneath Mount Etna and magma storage

A. D. L. Sharp*, P. M. Davis*[†] & F. Gray*

* University of Cambridge, Department of Earth Sciences, Bullard Laboratories, Madingley Rise, Madingley Road, Cambridge CB3 0EZ, UK

[†] University of California, Department of Earth and Space Sciences, 405 Hilgard Avenue, Los Angeles, California 90024

Travel time residuals and high frequency attenuation of regional and teleseismic events recorded at a seismic network around Mount Etna, Sicily, indicate the presence of a large volume of partial melt beneath the volcano. Petrographical evidence confirms that the anomaly is the main magma storage system and site of crystal differentiation beneath the volcano.

MOUNT ETNA, on the east coast of Sicily, is the largest and most active volcano in Europe. Hamilton¹ was one of the first to note that Etna did not have a simple, single eruptive site, but that the flanks of the volcano were covered with smaller eccentric cones. It was later^{2,3} deduced that there has been a NW migration of the main eruption centre with time. Imbo⁴ described Etna as having 266 eruptive systems, corresponding to the parasitic cones, spread over an area greater than 30 × 30 km on its flanks. This complex surface structure indicates large-scale subsurface features amenable to seismic investigation.

We installed a seismic network of short-period seismometers covering an area of 60 × 50 km around Mount Etna. The network was fully operational by the end of October 1977, and ran continuously until January 1978. The experiment used localized travel time delays of seismic waves from shots fired at sea, regional earthquakes and teleseisms, as well as waveform analysis to locate the source of Etna's lavas.

Background and geology

Mount Etna lies near the Calabrian arc and subduction zone, but on the side normally non-volcanic making its very existence surprising. The volcano is situated in a region of uplift on an E–W trending anticlinorium which is cut by the Messina–Comiso fault line. Parallel to this line are NNE directed normal faults having an easterly downthrow which suggests that E–W extension of eastern Sicily is responsible for the volcanic activity⁵. North of Etna, subduction beneath the Tyrrhenian Sea is demonstrated by the formation of the Aeolian Island arc. Chemically, however, Mount Etna is quite distinct from these island arc volcanoes, Etna being comprised of derivatives of true basaltic magmas (originating in the upper mantle) while the Aeolian Islands belong typically to the calc-alkaline series.

Petrological studies have revealed two main types of lava forming the volcano. The earliest etnean lavas are ~500,000 yr old, and are considered to be tholeiitic or transitional basalts, having a submarine character to the east, and a subaerial character to the west. These tholeiites are overlain by porphyritic differentiated alkali basalts which constitute the bulk of the volcano⁶. Although there have been periods of stronger differentiation, the overall composition of the differentiated alkali basalts has remained remarkably constant with time^{6–10}. Study of single lava flows has also revealed some variation in composition. Chemical differences have been observed between the first and last parts of the 1971 eruption lava, with little variation throughout the intermediate parts of the lava flow¹¹. This variation in chemistry of a single flow may indicate a secondary pneumatolytic differentiation within the feeding fissures in the upper volcanic layers before eruption^{7,11}. However, the source and evolution of the differentiated alkali

basalts are still undetermined. Rittmann¹² suggests that the chemical variation within single lava flows denies the existence of a single magma chamber beneath Etna, preferring a model of abyssal fissures in which crystal or pneumatolytic differentiation takes place, depending on the state of the vents as controlled by the movement of the surrounding crustal blocks. Cristofolini⁷ argues that the porphyritic nature and general uniformity of the lavas indicate strong gravitational settling of crystals and mixing with fresh magma in relatively large batches at intermediate depth. Variations within single flows are then explained by compositional gradients in the feeding fissures immediately before an eruption or from tapping different levels of the same chamber, or from tapping different chambers.

There have been few previous geophysical studies capable of investigating beneath the surficial layers of the volcano. Machado¹³ compared observed and theoretical seismic intensities of the Messina 1908 earthquake to obtain a large region of negative anomalies which were attributed to the absorption of seismic energy by a magma chamber under Etna. He deduced an approximately elliptical chamber, 40 × 100 km, assuming a mean depth of 5 km. No estimate of the vertical dimensions of the chamber could be made.

In 1968 a large refraction study was performed in northern Sicily¹⁴, the eastern section of the line running just to the north of Etna. Interpretation of the later arrivals on the record sections showed the eastern Sicilian coast to have a typical continental crust, but with low velocity zones in the range 9–24 km. The low velocity zones were attributed to higher temperature gradients caused by deeper magma chambers under Etna. However, the nature of the linear refraction profile prevented the lateral bounds of the anomaly being determined accurately. There is, therefore, good evidence for a body with low seismic velocity beneath the volcano.

The experiment

To investigate further the three-dimensional structure and physical properties of this anomaly, the seismic network was installed on Mount Etna with configuration shown in Fig. 1b. The network comprised four arrays each consisting of a base with a 14-channel tape recorder and a three-component set of short-period seismometers, together with three or four radio-linked outlying stations. Each outlying station had a single vertical short-period instrument.

Four shots were fired at sea (Fig. 1a) from the RV Shackleton to determine a crustal model for the area. The composite record section constructed from the shots showed clear first arrivals to greater than 160 km, but no correlatable later phases except a strong T-phase associated with the water-wave to P-wave conversion at the Messina Scarp off the east coast of Sicily. As the profile was unreversed, a simple horizontal-layered model could

be derived only, using the T-phase as a constraint on the uppermost layers. The crustal model so obtained is shown in Fig. 2, and is in good agreement with the simple structure derived from the reversed line of Cassinis *et al.*¹⁴.

Travel time residuals

The 14 earthquakes which were the clearest regional and teleseismic events recorded by the network were chosen for analysis (Table 1). These events were well distributed in azimuth (329–171°), range (1–10°) and depth (10–485 km), to give a broad distribution of rays with widely varying azimuth and angle of emergence passing through the crust beneath Etna. The average frequency content of the P-waves was 1.5–2.0 Hz, giving a resolution limit of ~4 km in the lower crust.

Using P-wave arrival times to infer crustal structure beneath a seismic network has been successful in determining low velocity bodies beneath Yellowstone, Wyoming^{15–17}, and other geothermal areas^{18,19}. By reducing the observed travel time ($t_{i,obs}$) from an earthquake to a station by a theoretical travel time ($t_{i,o}$), the residual

$$R_i = (t_{i,obs} - t_{i,o})$$

is formed. These residuals depend on the total ray path (or total travel time), source location and origin time. For any set of residuals $\{R_i\}$ from a single event, these dependencies may be removed by subtracting the mean value $\bar{R}$ of the residuals from each residual, to give the relative residuals

$$r_i = R_i - \bar{R}$$

The need for a reference station is thus removed, and the residuals will depend only weakly on the source parameters.

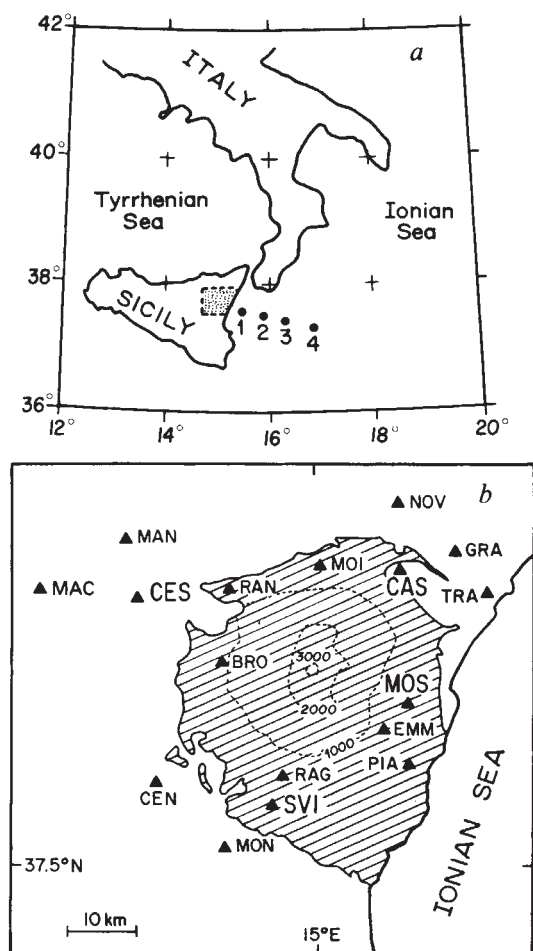


Fig. 1 *a*, Map of Southern Italy and Sicily showing the area covered by the seismic network (shaded), and shot positions (numbered). *b*, Seismic network configuration showing the four bases (CAS, MOS, SVI, CES) and the outlying stations. The shaded area defines the approximate extent of the etnean lavas.

The theoretical travel times were calculated using the US Geological Survey Preliminary Determination of Epicentre bulletins, bulletins of the Centre Seismologique Européo-Méditerranéen, and bulletins of the International Seismological Centre, to obtain source coordinates, and the travel time tables of Jeffreys and Bullen²⁰.

Wherever possible, the earthquake locations were compared with hypocentre redeterminations using Italian seismological observatory reports. A second set of residuals was obtained by subtracting from the arrival times the best fitting plane wave to the arrival times. The plane wave was calculated by the method of least squares with weighting dependent on the estimated accuracy of arrival time determination. The least squares residuals so obtained were generally in good agreement with those calculated from the theoretical travel times. Where the source location of an event was only poorly determined, the least squares residuals were used in the following analysis. Residuals from the arrival times of shots 2 and 3 were formed by calculating the theoretical travel times from the derived local crustal model.

Arrival times were obtained by visually measuring the first break of the P-wave from analogue records. Peaks, or later zero-crossing times^{21,19}, were not used as many signals showed increasing high frequency attenuation across the network. Such attenuation, due to lower Q paths, causes a relative broadening of the waveform which would give rise to errors in the timings of the peaks or zero crossings.

Examples of residuals are shown in Fig. 3. The shaded areas delineate approximately the areas of positive residuals (delays). All sets of residuals obtained exhibit similar features to those shown in Fig. 3, with the highest residuals always occurring on the opposite side of the volcano to the incident ray path, indicating the presence of a low velocity body beneath the array.

Several features of the low velocity anomaly may be qualitatively deduced from the general features of the delay patterns. The region of delay is of the order of tens of kilometres in lateral extent, and seems to be roughly ellipsoidal, elongated in a NNE direction. Also, the centre of each delay region seems to be displaced along the line of azimuth away from the centre of Etna, indicating that the anomaly lies at some depth beneath the volcano.

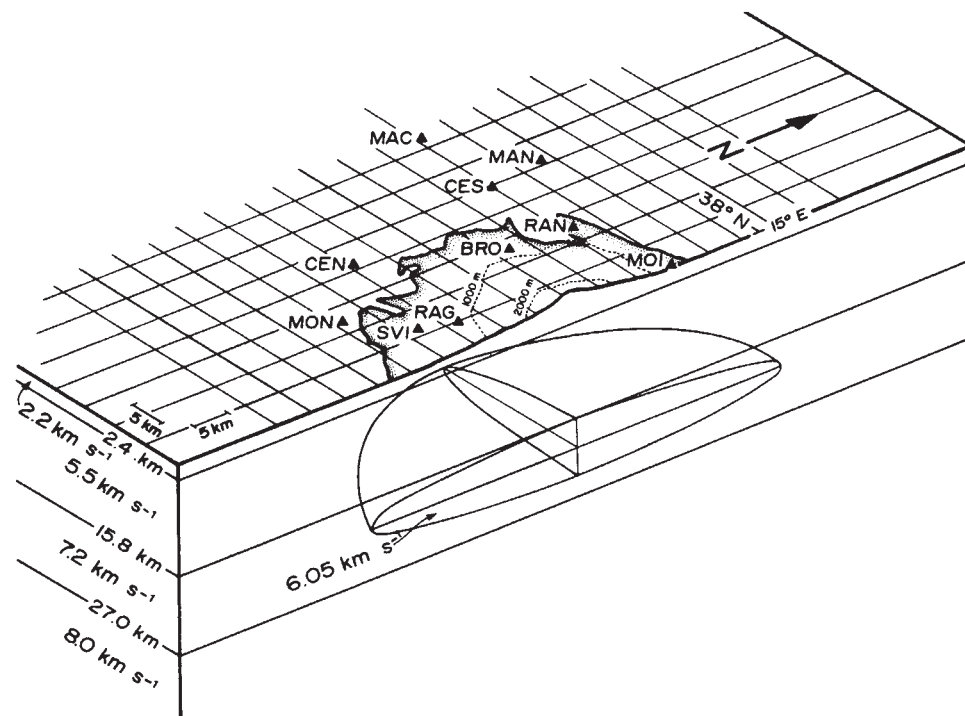
To quantify these observations, the low velocity anomaly was modelled as a tri-axial, ellipsoidal body centred at some depth and at some orientation beneath Etna, inverting for six parameters: ellipsoid axes, (a, b, c); depth to centre of ellipsoid, d ; orientation, θ ; and velocity reduction, δv (relative to the surrounding crustal velocity). Monte-Carlo inversion procedures are commonly used to invert nonlinear problems, but the random nature of the investigation may lead to poor sampling in parts of the parameter space. An organized grid search in the six-dimensional parameter space of the model was therefore adopted.

Over 20,000 models were tested by comparing the theoretical residuals with those observed. Theoretical residuals were calculated by ray-tracing through the combined crustal model and ellipsoidal anomaly structure for each ray, for each ellipsoidal model. As the velocity contrast was expected to be small, refraction effects at the boundaries of the ellipsoid were assumed to be negligible. The grid search was first performed with large grid intervals, then repeated with successively smaller intervals until the best model corresponding to the greatest reduction in the sum of squares of residual differences (observed – theoretical), $S(\phi)$, was obtained. The extent of the grid is given below, with the best model estimate in parentheses:

a	10–30	(22)	km
b	20–60	(31)	km
c	0–20	(4)	km
d	4–26	(20)	km
θ	5–40°	(13°)	
δv	–1.5––0.3	(–1.15)	km s ^{–1}

A sketch of the best model is shown in Fig. 2.

Fig. 2 Cut-away diagram of the crustal model and low velocity anomaly beneath Etna. Crustal layer velocities and depths are indicated. The ellipsoidal anomaly is shown centred beneath the volcano in the 7.2 km s^{-1} layer.



To estimate error limits on the best model, the criterion²²

$$S(\phi) \leq S(\hat{\phi}) \left\{ 1 + \frac{m}{n-m} f(m, n-m, (1-\alpha)) \right\}$$

was used, where n is the number of data and m the number of parameters. f is the value of the F -statistic for some confidence limit α , with m and $n-m$ degrees of freedom. $S(\phi)$ is the sum of squares of residual differences for a particular model, and $S(\hat{\phi})$ is the sum of squares of residual differences from the best model estimate. The six-dimensional parameter space surrounding the best estimate for all points satisfying the above criterion with $\alpha = 0.67$ was searched. The Hedgehog inversion procedure²³⁻²⁵ was adopted to select the acceptable models, being ideally suited to determining the confidence regions of nonlinear problems. The search for acceptable ellipsoid models was constructed within the grid:

a	0	(5)	50	km
b	0	(5)	80	km
c	0	(2)	30	km
d	3	(4)	40	km
θ	0°	(5°)	60°	
δv	-4.0	(0.2)	-0.1	km s^{-1}

The above list shows the limits of the search, with the grid intervals in parentheses. Using the Hedgehog procedure, only those points in the neighbourhood of successful models were tested to give a singly-connected space of acceptable models. The set of acceptable models is shown in Fig. 4. Values for the a -axis and depth of the ellipsoid seem to be well constrained as no acceptable models were found with values for a and d other than $a = 22 \text{ km}$ and $d = 20 \text{ km}$. Thus, only the variation of the other four dimensions need be examined. This variation is shown in the four-dimensional plot of Fig. 4. From each of the small sets of axes (c -axis versus δv), a trade-off between the height of the ellipsoid (c -axis) and the relative velocity reduction (δv) is seen. The large variation in values for the b -axis may be attributed to the network not being of sufficient extent in a N-S direction to cover the delay anomaly fully. This lack of resolution in the b -axis may also have caused some of the scatter in the determination of the orientation, θ , of the ellipsoid. The well resolved depth parameter confirms the existence of the deep low velocity zone of Cassinis *et al.*¹⁴, and does not admit the possibility that the delay effects may be of upper crustal origin.

The above modelling has determined the ellipsoidal model, and associated error limits, which are the most consistent with the data. However, the single ellipsoidal model does not

Table 1 Shot and earthquake parameters

Date	Origin time (UTC)	Epicentre	Depth (km)	Distance (deg)	Azimuth (deg)	
1 November 1977	1238:15.2	37.47° N 15.86° E	0	0.7	112	(Shot 2)
1 November 1977	1538:20.5	37.37° N 16.27° E	0	1.1	110	(Shot 3)
8 November 1977	2144:46.4	34.55° N 15.59° E	10	3.2	171	CSEM
26 November 1977	1319:50.8	38.64° N 20.37° E	48	4.3	76	CSEM
28 November 1977	0259:10.0	35.96° N 27.79° E	74	10.4	96	ISC
3 December 1977	0539:29.5	40.25° N 19.90° E	27	4.6	55	USGS
8 December 1977	0040:43.6	35.21° N 23.38° E	62	7.2	108	USGS
9 December 1977	1553:40.5	38.37° N 27.35° E	33	9.7	83	CSEM
15 December 1977	0806:10.8	34.92° N 23.08° E	47	7.1	111	USGS
16 December 1977	0737:32.3	38.45° N 27.36° E	36	9.7	82	CSEM
17 December 1977	0546:45.0	34.60° N 15.90° E	33	3.2	167	ISC
20 December 1977	2004:18.6	38.58° N 15.66° E	196	1.0	32	CSEM
25 December 1977	0211:12.5	40.38° N 12.95° E	485	3.1	329	CSEM
30 December 1977	1735:3.2	40.11° N 16.42° E	306	2.6	25	Relocated
30 December 1977	1808:51.3	39.97° N 16.00° E	270	2.5	19	Relocated
12 January 1978	2008:40.6	36.00° N 22.26° E	49	6.1	105	CSEM

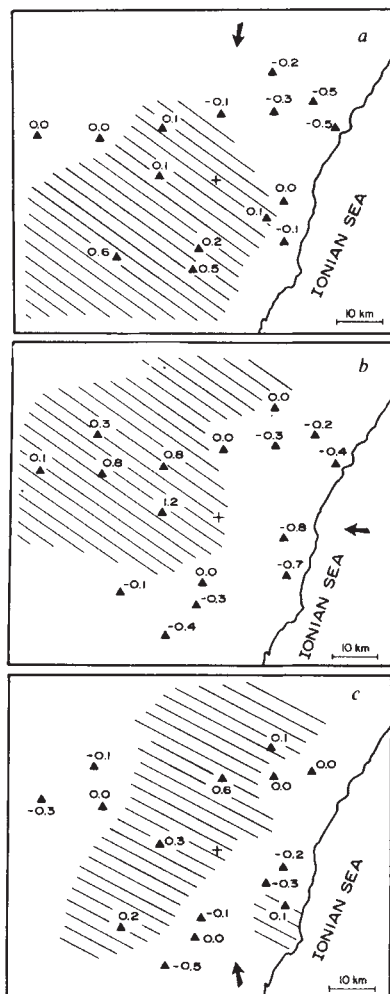


Fig. 3 Examples of travel time residuals from three earthquakes occurring: *a*, To the north, 30 December 1977 18 h 08 min; azimuth = 019° ; $\Delta = 2.5^\circ$; $d = 270$ km. *b*, To the east, 28 November 1977 02 h 59 min; azimuth = 96° ; $\Delta = 10^\circ$; $d = 74$ km. *c*, To the south, 8 November 21 h 44 min; azimuth = 171° ; $\Delta = 3^\circ$; $d = 10$ km. Hatching shows areas of positive residuals. Arrows indicate the azimuths of the incoming waves as observed by the network.

completely fit some of the highest delays shown in Fig. 3. These large delays may indicate a secondary velocity anomaly or a non-uniformity in the present simple ellipsoidal velocity anomaly. But the present data are insufficient to resolve such finer structures.

High frequency attenuation

Many signals exhibited loss of high frequencies across the network. Figure 5 shows the P-wave arrival of the same earthquake as recorded by two stations. The waveform TRA has travelled from the source to the station without passing through the low velocity anomaly, whereas the waveform MAN has passed through the anomaly and has been attenuated in high frequency relative to TRA. Plotting the logarithm of the spectral ratio against frequency, the reduction in high frequency content of MAN is even more evident (Fig. 5). The gradient of the line may be related to the attenuation factor²⁶ Q . Assuming an average Q of 50 for the crustal layers surrounding the ellipsoid anomaly, a Q of 9 must be assumed for the anomaly itself to account for the high frequency attenuation.

Interpretation and discussion

Reduction in P-wave velocity may be caused by several factors (for example, a crack system, or simply a rigid body of lower seismic velocity). However, it is doubtful whether an open crack system can be maintained at depths of 20 km (equivalent to

pressures of ~ 6 kbar). The large reduction of high frequencies (low Q) as shown by waveforms passing through the anomaly implies that the body is not completely rigid. This agrees with Machado's model of a magma chamber¹³.

The simplest explanation of the observed results is that the ellipsoidal anomaly is the seismic manifestation of the main crustal magma storage region beneath Etna. The velocity reduction within the anomaly is 16% indicating that the ellipsoid is not completely molten. Assuming the body to be a partial melt composed of solid (7.2 km s^{-1}) and melt (3.0 km s^{-1}), the percentage of melt within the body is found to be 14%—corresponding to $1,600 \text{ km}^3$ of melt in a total of $11,424 \text{ km}^3$. Thus the magma chamber is not completely molten in the 'classical' sense, but rather it is likely to be formed by a complex series of veins (or fissures) of melt running through the ellipsoidal body.

Geological evidence supports these conclusions. The results of Cristofolini⁷, and Romano and Sturiale¹¹ require that there must be a large magma storage system beneath Etna in which differentiation (gravitational crystal settling) takes place. Although Rittmann¹² argues that there is no single great magma chamber, his model of Etna does require differentiation of parental magma within "abyssal fissures". Thus the two geological models are not completely dissimilar, both requiring the temporary storage of magma at some depth.

The new geophysical model, although very approximate, demonstrates that there is a substantial quantity of magma stored beneath the volcano, and that the storage mechanism is more likely to be in a series of (interconnecting) fissures, rather than a single, completely molten chamber. The orientation and elongation of the geophysical model also agrees with the regional tectonic dislocations, N-S and NE-SW⁶, supporting the hypothesis that etnean volcanism is related to lithospheric extension in that area^{7-12,27}.

Mineralogical studies^{8,9} show that plagioclase is the dominant phenocryst material of the trachybasalts, having 20–30% by volume of plagioclase phenocrysts. Taking 'pyrolite'²⁸ as the parental material, Duncan²⁹ showed that fractional crystallization producing plagioclase phenocrysts must occur at depths less than 25 km. Hammill³⁰ also showed that differentiation trends in the fractional crystallization of high alumina clinopyroxene and titaniferous magnetite indicated differentiation at pressures of ~ 10 kbar. These findings agree well with the geophysical model of partial melt in the depth range 16–24 km.

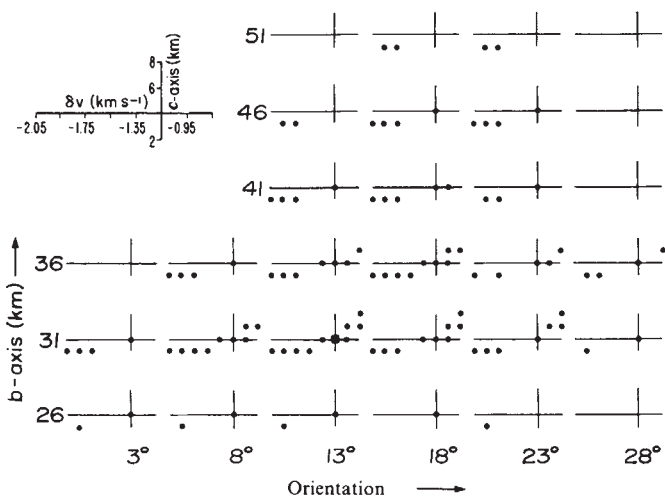


Fig. 4 Four-dimensional plot of all acceptable models from Hedgehog inversion. The small pairs of axes represent the velocity reduction δv (abscissa) and ellipsoid c -axis (ordinate), as shown in the inset. Each axes pair has an associated value of orientation θ , and ellipsoid b -axis which are given on the large horizontal and vertical axes, respectively. ■, The 'best' solution. As no acceptable model was found with a -axis and depth values other than $a = 22$ km and $d = 20$ km these may be regarded as constants associated with each of the plots.

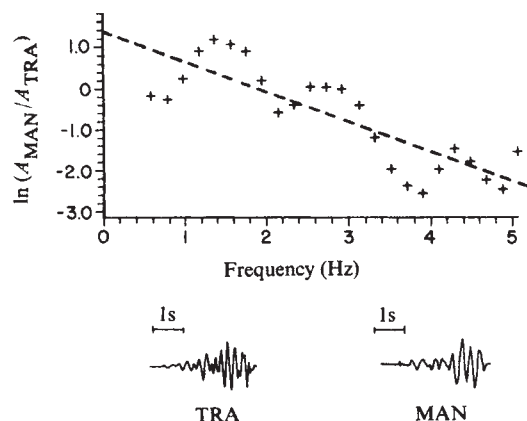


Fig. 5 P-wave arrivals from a single earthquake as recorded by two stations. The waveform TRA has not travelled through the anomaly, whereas the waveform MAN has passed through the anomaly and exhibits a loss in high frequency content relative to TRA. The plot shows the logarithm of the spectral ratio of the two signals, $\ln(A_{MAN}/A_{TRA})$, plotted as a function of frequency. The average slope indicates a difference in the Q values of the two paths. Minor deviations from the average slope may be attributed to small differences in the crustal structure at the two stations.

It is also significant that the local etnean micro-earthquakes have a maximum depth³¹ of ~12 km, suggesting that they occur in a brittle region overlying the volume of partial melt.

Conclusions

From a seismic network study of the etnean volcanic region, anomalous P-wave delays and high frequency attenuation of seismic signals have been observed. Simple three-dimensional

modelling and inversion procedures show that the anomaly can be well represented by a triaxial ellipsoid of axes 22 km, 31 km (horizontal) and 4 km (vertical), at a depth of 20 km below Etna. The ellipsoid is elongated in a NNE direction, similar to the regional tectonic dislocations, and the velocity reduction of 16% indicates that the anomaly is most likely to be a series of fissures containing melt (14% by volume), rather than a completely molten chamber.

We conclude that lithospheric extension of eastern Sicily allows basic parental magma to rise from the upper mantle which is then trapped in a complex series of fissures at depths of 16–24 km, just beneath the 5.5/7.2 km s⁻¹ discontinuity. Crystal fractionation, including some mixing with the parental magma, takes place within the fissures⁷. The differentiated magma then ascends into higher feeders, generally along the lines of tectonic dislocation, where secondary pneumatolytic differentiation takes place before eruption, causing minor fluctuations in the chemistry within individual lava flows¹¹.

Small variations in the differentiates with time may either be due to a change in the supply of parental magma with time, or a change in the time during which the magma is stored in the fissure system. The latter could be controlled by the tectonic movements of the surrounding crustal blocks¹².

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Agulhas Basin magnetic bight

H. W. Bergh & D. M. Barrett*

Bernard Price Institute of Geophysical Research, University of the Witwatersrand, Johannesburg, South Africa 2001

The discovery of a magnetic bight in the Agulhas Basin places unique constraints on the relative motion between South America, Antarctica and Africa in the late Cretaceous.

RECONSTRUCTIONS of the predrift configuration of the three Western Gondwana continents (South America (SAM), Africa (AF) and Antarctica (ANT)) have relied heavily on the matching of selected bathymetric contours. Only in fitting AF against SAM and the northern edge of the Falkland Plateau has tightly constraining geophysical evidence been available. This sort of evidence (from fracture zone trends and recognition of linear

magnetic anomalies) is only sparsely available for the separation history of the AF and ANT plates.

Well-documented Cretaceous and Eocene magnetic anomalies have been found¹ in the Mozambique Basin eastward of the Prince Edward Fracture Zone. A Mesozoic magnetic anomaly sequence in the Antarctic Basin² points towards a simple history of ANT–AF separation, while recently reported^{3,4} Mesozoic anomalies in the northern Mozambique Basin provide evidence for the same simple manner of seafloor generation west of the Prince Edward Fracture Zone. No direct determinations have

* Present address: Esso Minerals (Africa) Inc., PO Box 78011, Sandton, Johannesburg, South Africa 2146.

been made of past relative motion between SAM and ANT.

We describe here results from a geophysical survey of a small area of deep ocean south-west of the Agulhas Plateau (see inset, Fig. 1, for area location). Within this region we can clearly recognize an Upper Cretaceous magnetic bight at two points in time. The new data enable us to describe the behaviour of the triple junction SAM-AF-ANT at ~80 Myr ago. Previously unpublished data in the Antarctic Basin, combined with our new findings, are used to reconstruct the relative SAM-AF-ANT geometry at that time.

Magnetic bight data

Figure 1 shows total magnetic field anomalies plotted perpendicular to ship's track on a bathymetric chart of the survey area. The magnetic measurements were made from the MV RSA during October 1977. Bathymetric coverage on that cruise was supplemented by data from Lamont-Doherty Geological Observatory (Vema and Conrad) and by one Atlantis II track⁵ run by the Woods Hole Oceanographic Institute.

Seafloor relief south-west of the Agulhas Plateau is generally small and sediment cover thin (between 0.0 and 0.5 s of two-way travel time). The slight elevation around 45°S, 20°E, called the Southwest Spur⁶, appears to be partly due to raised basement and partly to increased sediment thickness. The deeper region between here and the Agulhas Plateau is heavily scoured, with

scattered erosional outcrops hinting at a possible linear continuity of elevated basement.

The magnetic anomalies clearly delineate a chevron-shaped strip of negatively magnetized sea floor even where this is right-laterally offset in the south-east. The two limbs of this magnetic bight strike 150° (north-west limb) and 125° (south-east limb). We interpret these two limbs as being respectively related to relative motion between the plate pairs SAM-AF and ANT-AF. Correlation of the magnetic anomalies with the geomagnetic reversal time scale is based on the findings between Antarctica and Africa. Recent data between these two continents⁷ show clearly that magnetic anomaly sequences are symmetric about the present spreading axis at 52.5°S, 18°E and almost complete out to anomaly 34 on either side. In Fig. 1 the northernmost anomalies are numbered, 30–34, of this complete sequence. The region with dotted negative anomalies corresponds to sea floor generated between anomaly 33 and 34 time. In terms of a recent reversal time scale⁸ this refers to the period between 79.6 and 76.5 Myr ago. It corresponds to the A-section of the proposed type-section⁹ from Gubbio in Italy.

Unique identification of magnetic anomalies on the north-west limb of the bight depends on their continuity with the same anomalies on the south-east limb as we are unable to recognize clearly identifiable anomalies immediately younger than 33 between South America and Africa. This is partly because of

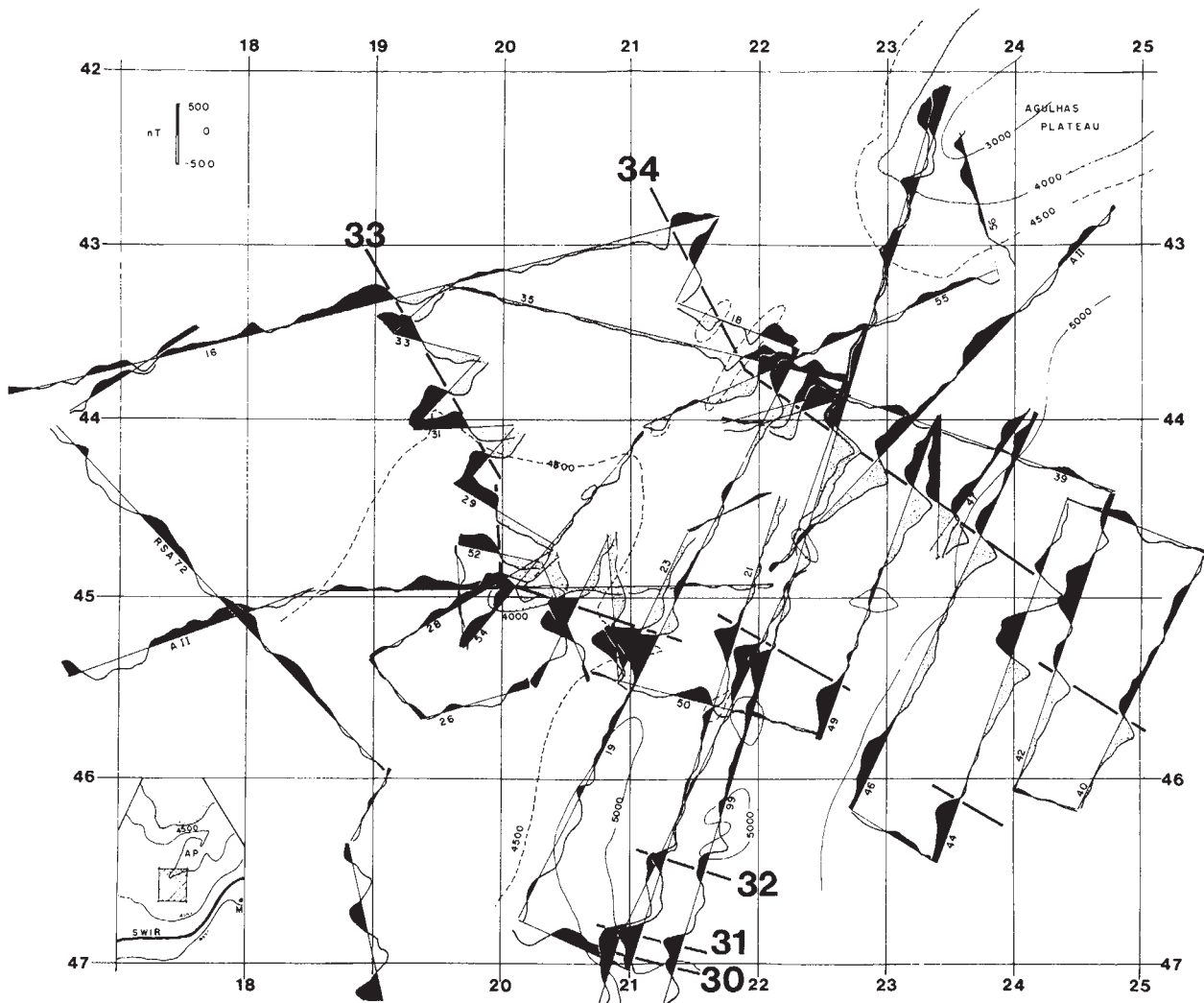


Fig. 1 Magnetic anomalies (total field minus IGRF 1975.0) plotted perpendicular to ship's tracks. Lines marked consecutively from 16 to 56 were run in October 1977 aboard the MV RSA. Lines 99 and RSA 72 were run between Antarctica and South Africa on the RSA in 1977 and 1972 respectively. AII is an Atlantis II track⁵. Positive magnetic anomalies are in black and the lines 34 and 33 refer to the end and beginning of the relevant polarity epochs. The inset shows the survey area in relation to southern Africa, the Agulhas Plateau (AP) and the Southwest Indian Ridge (SWIR). Bathymetry in metres.

poor data coverage but is also probably due to the complications of a westward ridge jump south of the Agulhas–Falkland Fracture Zone soon after anomaly 33 time. Such a jump has been postulated^{10,11} to account for the present ridge offset across this fracture zone being ~1,150 km less than its probable offset at the initiation of relative motion between SAM and AF. The timing and nature of this ridge jump, or jumps, are still highly speculative.

Average spreading rates on the two limbs of the bight were determined for the negative polarity interval by projecting the magnetic anomalies perpendicular to their average strike (Fig. 2). In determining the latter parameter, the bight apex portion of anomaly 33 was neglected because it appears atypical of the general trend. We assume here that the anomalies are perpendicular to the spreading direction at their time of formation. This assumption seems to be appropriate^{12,13} for spreading half-rates greater than about 30 mm yr⁻¹. Comparison of the projected profiles with suitable simulated profiles (Fig. 2) yields spreading half-rates of 40 mm yr⁻¹ for ANT–AF and 55 mm yr⁻¹ for SAM–AF.

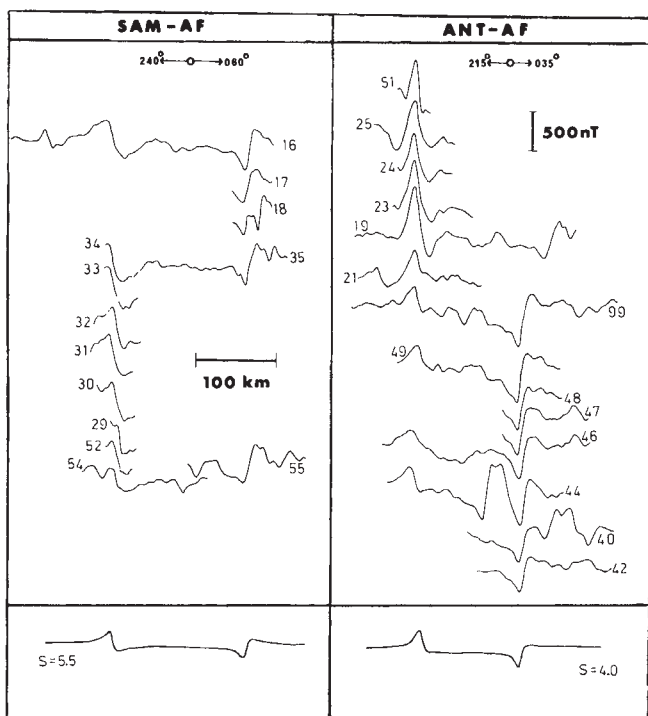


Fig. 2 Magnetic anomaly profiles projected perpendicular to the average strike of anomalies 33 and 34. The simulated profiles at the bottom give the spreading half rates S in cm yr⁻¹ for the two plate pairs.

The ridge sections which generated the observed magnetic bight formed two arms of a triple junction at anomaly 34 time. For this junction to be stable the third arm must also have been a ridge. An RRF (ridge–ridge–fault) triple junction could only be stable if the two ridge segments were at right angles¹⁴ or at least one ridge was very oblique to the spreading direction. From the velocity triangle (Fig. 3) we see that the SAM–ANT boundary was a ridge trending slightly east of north (with respect to a fixed AF) and having a half-spreading rate of 25 mm yr⁻¹.

80-Myr reconstruction

Figure 4 is our best reconstruction of the western Gondwana continents at anomaly 34 time. The position of South America relative to Africa differs insignificantly from published palaeo-locations^{15,16}. Antarctica's palaeoposition has been constrained

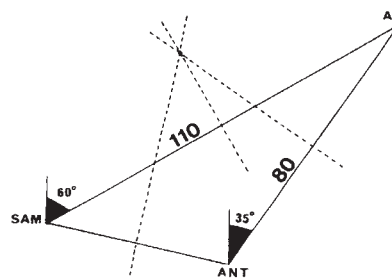


Fig. 3 Velocity triangle for the SAM–AF–ANT triple junction between anomaly 34 and 33 time. Angles give the azimuths of AF–SAM and AF–ANT relative motion and the total separation rate is shown in mm yr⁻¹.

by our recent findings in the Antarctic Basin and south-west of the Agulhas Plateau and agrees closely with that predicted by Norton and Slater¹⁷.

With Africa kept fixed, we have rotated South America to the Bullard *et al.*¹⁸ fit and then applied a clockwise rotation of 28°, using the LePichon and Hayes¹⁵ rotation pole at 22°N, 14°W. This rotation is only 2° more than theirs and is justified by the excellent matching of previously unpublished magnetic anomaly 34 findings in the Cape Basin with the corresponding anomalies¹⁶ in the Argentine Basin. The Cape Basin anomalies, shown in Fig. 5a, are the best evidence we have for the location and strike of anomaly 34 immediately north of the Agulhas–Falkland Fracture Zone.

The Antarctic rotation pole was found by matching of anomalies 34 on either side of the Southwest Indian Ridge.

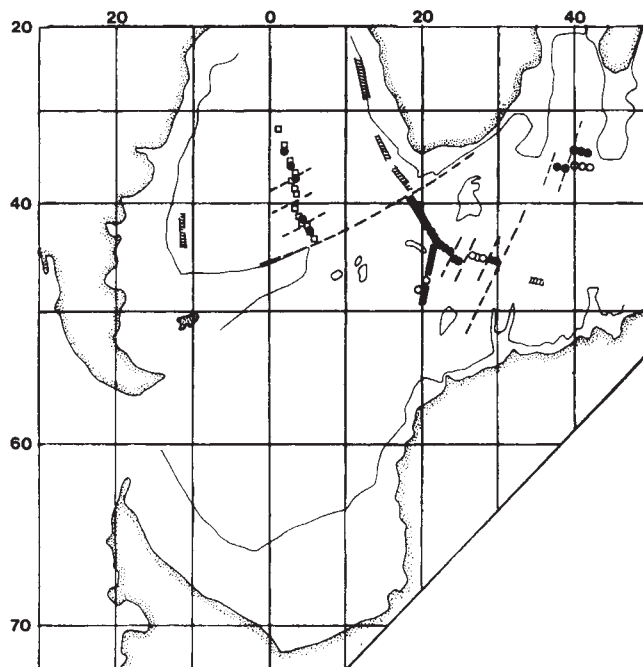


Fig. 4 80-Myr reconstruction of western Gondwana continents. Africa has been kept fixed. Anomaly 34 is shown as: ●, on African plate; □, rotated with SAM; ○, rotated with ANT. The rotations are described in the text; ANT has been rotated by 18° about a pole at 10°N, 40°W. Sea floor formed in the Mesozoic interval defined by anomalies M2 to M4 is hatched. Fracture zones are marked by dashed lines, the 3,000-m isobath by a thin solid line. No significance should be attributed to the retention of Madagascar in its present position.

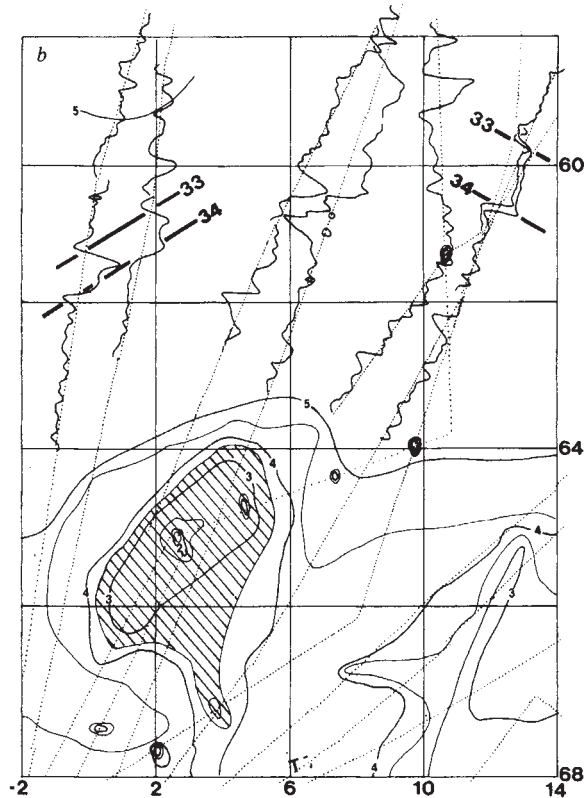
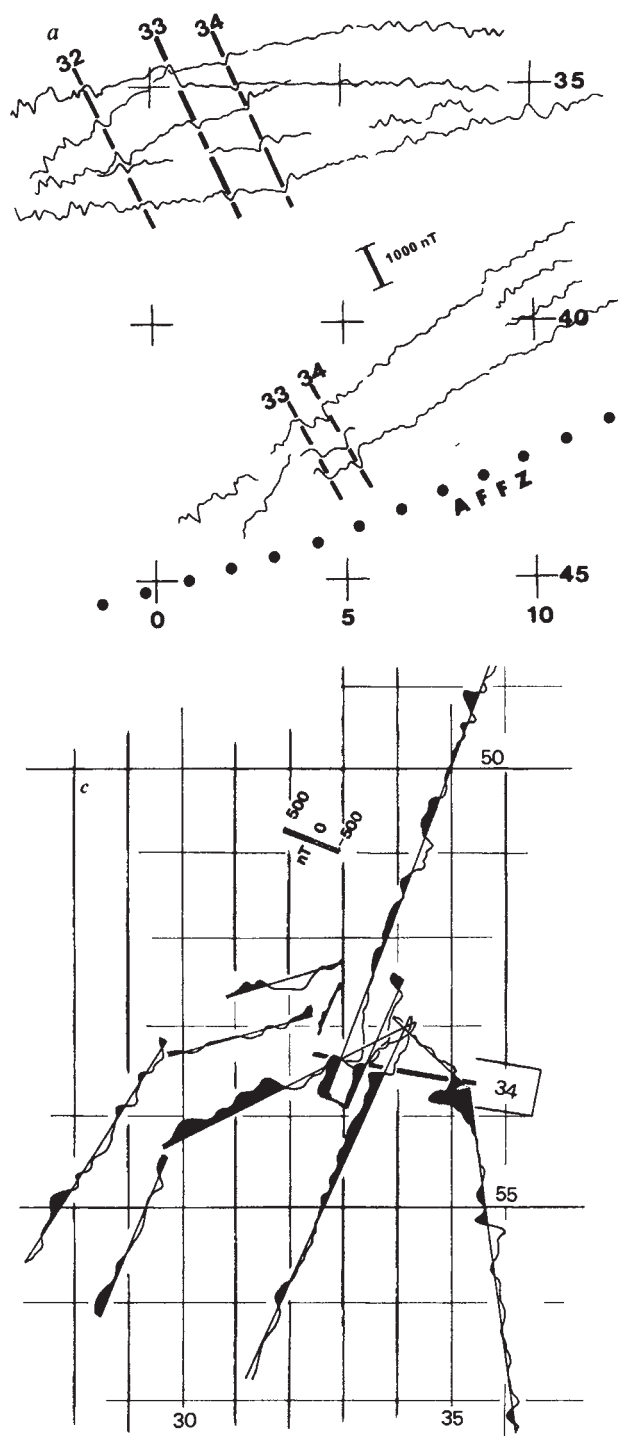
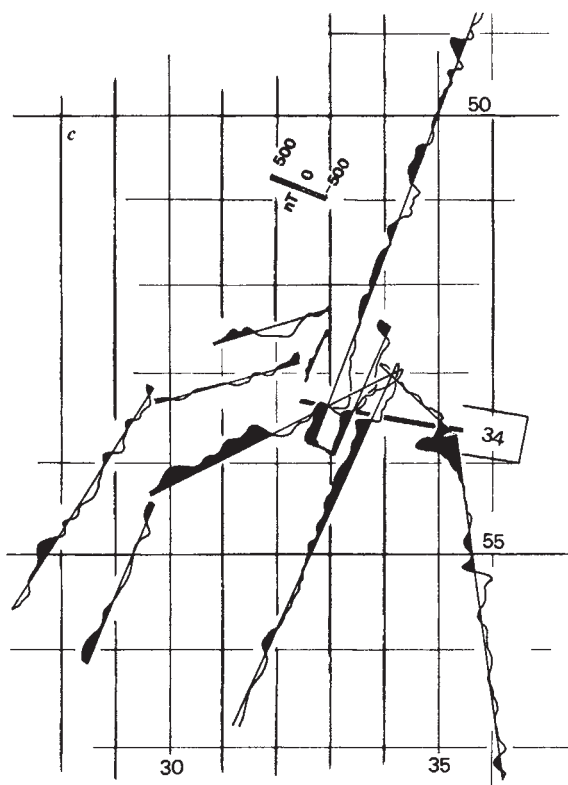


Fig. 5 Unpublished magnetic anomaly profiles obtained during voyages of the MV RSA: *a*, in the South Atlantic immediately north of the Agulhas Falkland Fracture Zone (AFFZ); *b*, north of Maud Rise; *c*, near 54°S, 34°E.



There is uncertainty over whether these conjugate anomaly pairs were formed at exactly the same section of spreading ridge. This need not be too serious a drawback as long as the relevant ridge segments did not have large offsets from one another. We used a very definite anomaly 34 north of Maud Rise (Fig. 5*b*) and a more tentatively identified one at 54°S, 34°E (Fig. 5*c*). The pole and angular rotation were adjusted to bring these into best coincidence with, respectively, the anomalies reported here and those in the Mozambique Basin¹.

The pole used for the Antarctic rotation is not much different from the present pole¹⁹ and supports the suggestion² that the average ANT-AF pole since the Mesozoic closely approximates the present one. An interesting feature of Fig. 4 is that our questionable SAM-ANT spur of the anomaly 34 right (Fig. 5*b*)

rotates into the predicted position of the SAM-ANT ridge axis at the time of the reconstruction. More intense magnetic surveying is planned near Maud Rise to check on the inferred Antarctic Basin Bight.

Discussion

We confine ourselves here to the evolution of the triple junction between anomaly 34 and 33 time and to possible changes in ANT-AF relative motion immediately after that period. The implications of the striking geometrical relationship between the Agulhas Plateau and the fossil triple junction will be considered elsewhere.

At anomaly 34 time the triple junction was probably a simple RRR with fast orthogonal spreading from all three limbs. By 33 time the ANT-AF half rate had commenced slowing down drastically to $\sim 11 \text{ mm yr}^{-1}$, as determined from the spacing between anomalies 30 and 32. The simultaneous change in SAM-AF relative motion is well documented¹⁶. Changes in the vicinity of the triple junction seem to be dominated by the removal of the orthogonality constraint as the spreading rates were reduced. Two new transform faults were created and the ridge sections meeting at the triple junction rotated to reduce the lengths of these transforms, as seems to occur generally¹². The azimuth of the ANT-AF ridge section immediately adjacent to the triple junction is preserved in later linear magnetic anomalies to the south. This incorporation of a persistent oblique spreading regime occurred at the same time in the Mozambique Basin¹ and thus seems to represent a stable mode for slow separation of two plates.

We recognize here that representative spreading rates are imprecisely determined from observations on one plate only of anomalies formed near a rapidly reorientated plate boundary. The problem is more severe near a triple junction, because three relative velocity vectors are changing sympathetically. The

SAM-AF velocity determined from the north-west limb of the bight is based on an assumption of orthogonal, symmetric spreading. The limb strikes very nearly at right angles to the Agulhas-Falkland Fracture Zone (AFFZ). However, asymmetric SAM-AF spreading in the late Cretaceous has recently been proposed²⁰ immediately south of this fracture zone, and is also apparent when the anomalies in Fig. 5a are compared with those near 45°S on the South American plate¹⁶. We do not consider the variation in total SAM-AF separation rate to be sufficiently well known at that time to justify much discussion here. The effect of dominant accretion on the AF side of the SAM-AF boundary would have been to increase progressively the obliqueness of the SAM-ANT (ridge) boundary. The ANT-AF velocity seems to be on the high side as the Mozambique Basin half-rate¹ (at 90° from the rotation pole) is 35 mm yr⁻¹ using the same time scale⁸ as in the present paper. A more consistent half-rate (32 mm yr⁻¹) is obtained using the extreme south-east section of the bight. The effect on SAM-ANT spreading geometry, of taking this possible error into account, would be to make the ridge section more nearly north-south, with a slightly higher spreading rate.

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The velocity triangle in Fig. 3 shows that the RRR triple junction will migrate northwards, with respect to ANT, along the SAM-AF rifting margin. Ridge jump proposals^{11,20,21} have not considered the proximity of a triple junction to the AFFZ.

A stable RRF junction could possibly be formed if the RRR triple junction reaches this fracture zone. Such a junction with almost orthogonal SAM-ANT and AF-ANT ridges would move away from AF at half the SAM-AF velocity and so would not reduce the active transform length of the AFFZ. If oblique spreading is invoked, it would be possible for the RRF triple junction to migrate towards the SAM-AF ridge section north of the AFFZ, but not at sufficient speed to cause the large reduction in offset since the Cretaceous. Detailed surveying south of the AFFZ is still needed to resolve this problem.

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X-ray kinetic studies of microtubule assembly using synchrotron radiation

Eva-Maria Mandelkow, Arnold Harmsen & Eckhard Mandelkow

Department of Biophysics, Max-Planck-Institute for Medical Research, Jahnstrasse 29, D-6900 Heidelberg, FRG

Joan Bordas

European Molecular Biology Laboratory, c/o DESY, Notkestrasse, D-2000 Hamburg 52, FRG

The assembly of microtubules has been investigated by time-resolved X-ray diffraction using synchrotron radiation. The small-angle scattering becomes visible within seconds and thus enables study of the structural transitions of the protein aggregates during assembly from their subunits in solution. The X-ray pattern at 4 °C arises from a mixture of tubulin rings, dimers and some other species. Raising the temperature to 36 °C induces the breakdown of rings, followed by the growth of microtubules. The results suggest that microtubules may be formed from tubulin oligomers smaller than rings.

WE report here a study on tubulin polymerization and depolymerization *in vitro*, induced by variation of temperature and monitored by time-resolved X-ray scattering. The aims of our experiments were (1) to establish the usefulness of a new approach to the study of polymerization processes in general, and (2) to understand better the stages of microtubule assembly. We investigated the type of structural entities present in the several stages of assembly and disassembly, their time dependence, their dependence on chemical conditions, and the possibility of detection of intermediate states not previously encountered.

Equipment and method

The technique uses high flux of X-rays available from the positron storage ring DORIS at Hamburg, suitable X-ray optics, detector and data acquisition system¹⁻³ and offers the

advantage of yielding not only the rate constants of the process under study, as for instance light scattering or turbidity measurements might do, but also time-resolved structural detail, comparable and/or complementary to that provided by static X-ray diffraction or electron microscopy. During the measurements the accelerator was running at 5 GeV with currents ranging over 10-25 mA. Typical fluxes on the specimens were of the order of 5×10^{10} photons s⁻¹. The wavelength of the radiation was 1.5 Å. Temperature control was achieved by containing the sample in a thermostatted cell around which circulation of a cold or warm fluid could be quickly selected by a system of switching valves.

The microtubule protein (tubulin and microtubule-associated proteins (MAPs)⁴) was prepared from porcine brain by temperature-dependent cycles of assembly and disassembly without glycerol⁵.

Experimental results

Figure 1 shows the behaviour of the system and corresponds to a three-dimensional plot, with the scattered intensity in the vertical axis, and the scattering angle and time course in the two horizontal axes.

We used temperature jumps from 4 to 36 °C and vice versa to induce protein assembly and disassembly. The half time of the jump was around 25 s (heating rate $0.6\text{ }^{\circ}\text{C s}^{-1}$). Two successive cycles of polymerization and depolymerization are depicted. The most prominent changes in the scattering patterns are in the region of the central scatter and in the positions and heights of the subsidiary maxima. The change of one type of scattering pattern into another, following the T-jump, is complete within ~150 s. In spite of the high flux of X-rays the polymerization properties are not affected noticeably by radiation damage

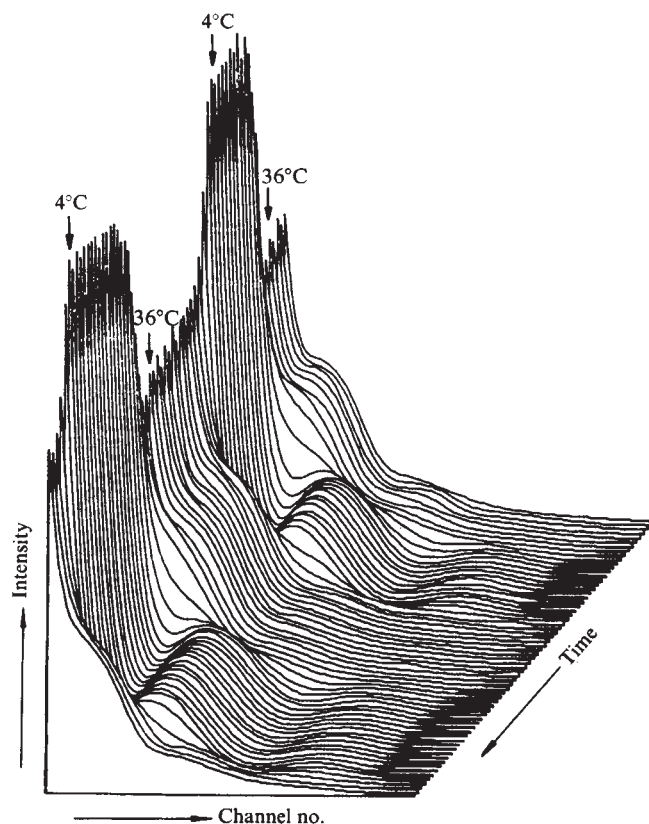


Fig. 1 Composite view of scattering traces obtained during two cycles of polymerization. Intensities were accumulated over time frames of 15 s, but only alternate frames are shown here. Temperature changes are as indicated. The half time of the T-jump is 25 s. Specimen-detector distance 320 cm, detector bin size 0.31 mm (80 mm per 256 channels). Note the changes in the height of the central scatter (left) and in the positions of the subsidiary maxima. The central scatter terminates abruptly due to a lead beam stop in front of the detector. The curves have been corrected for counter response, beam fluctuations, background, and splined to eliminate statistical noise. Microtubule protein was prepared from porcine brain following Borisy *et al.*⁵. The reassembly buffer was usually 0.05 M or 0.1 M PIPES, 1 mM each of GTP, dithiothreitol (DTT), EGTA, and MgSO_4 , pH 6.94 at room temperature. After the second cycle the protein pellets were stored in liquid nitrogen. They were redissolved in reassembly buffer before use and either used directly or run through one additional cycle. Protein determinations were done by the Lowry assay. The efficiency of assembly was monitored turbidimetrically, and the protein composition was checked by SDS-polyacrylamide gradient gel electrophoresis and analytical ultracentrifugation.

during the course of an experiment. Thus the cycles of assembly and disassembly can be repeated several times as long as GTP is in excess of the GDP formed during assembly.

Figure 2 shows four scattering traces selected from Fig. 1. They correspond to the initial scattering from the cold solution, two intermediate states which appear shortly before the onset of microtubule formation, and the final pattern at high temperature. There is an overall decrease of the scattered intensity when going from the initial cold (Fig. 2a) to the first intermediate state (Fig. 2b). The subsidiary maxima around channels 12, 41 and 65 characteristic of the cold state are reduced. This is followed by a second intermediate state in which the subsidiary maxima are washed out and the central scatter increases again (Fig. 2c). The scattering of the final warm state (Fig. 2d) shows a marked increase near the origin as well as the appearance of new subsidiary maxima around channels 26 and 72.

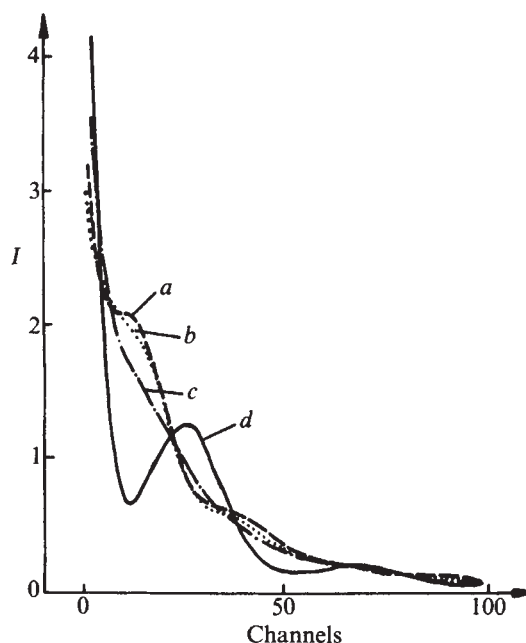


Fig. 2 Selected plots derived from Fig. 1. *a*, Initial cold solution, showing subsidiary maxima around channels 12, 41 and 65. *b*, 45 s after the temperature switch. At this point the temperature in the solution is about 27 °C. It corresponds to the bottom of the undershoot of Fig. 3b. The integrated intensity of the central scatter has decreased by about 8%, and the subsidiary maxima are reduced. *c*, 75 s after T-jump, corresponding to the recovery of the undershoot of Fig. 3b. The central scatter has increased about 8% over its initial value, and the subsidiary maxima have disappeared. *d*, Final warm solution, 225 s after T-jump. The central scatter has increased by 45% over the initial value. New subsidiary maxima appear further away from the origin, around channels 26 and 72.

Figure 3 shows the time course of the temperature in the specimen with the integrated intensities in several regions selected from Fig. 2. The central scatter (Fig. 3b) shows an undershoot which reaches its minimum value 45–60 s after the T-jump. This is followed by a sharp increase with a half time of ~100 s. The bottom of the undershoot corresponds to the intermediate scattering pattern shown in Fig. 2b. When the temperature is switched back from 36 to 4 °C, a continuous, biphasic, downward trend is observed.

The intensity integrated between channels 11 and 21 (first subsidiary maximum of the cold solution, Fig. 3c) decreases in a biphasic manner. The first phase coincides in time with the appearance of the undershoot in the central scatter, the second with its steep increase (Fig. 3b). The integrated intensity around channels 22–32 (first subsidiary maximum of the warm solution,

Fig. 3d) follows a similar trend to the central scatter, but there is an additional lag of 30 s in the polymerization phase. During the depolymerization following the downward T-jump, the intensities return to their approximate initial values, but the lag and half times are shorter than for polymerization.

Figures 1–3 suggest that the assembling system responds to the temperature jump in at least two phases. The first is characterized by a general decrease of intensity throughout the angular range observed. During the second phase the intensity rises again in some regions (Fig. 3b, d) but continues to drop in others (Fig. 3c). This classification is probably oversimplified as the transition between the phases does not occur simultaneously in all parts of the pattern.

The difference between the two main phases may be demonstrated more clearly by reducing the heating rate to about $0.02\text{ }^{\circ}\text{C s}^{-1}$. In this case the system is much closer to equilibrium than in a T-jump experiment. Nevertheless, the time courses of the intensities are surprisingly similar in both conditions. Figure 4 shows initially a slow drop of intensity in all regions of the pattern with rising temperature. Around $25\text{ }^{\circ}\text{C}$ there is an abrupt transition—the intensity in Fig. 4b and d rises again while that of Fig. 4c drops steeply.

Correlation with physicochemical experiments

The interpretation of X-ray scattering from proteins in solution usually depends on additional experiments to distinguish between different model structures. Gel electrophoresis shows the microtubule protein to consist of tubulin (α and β , $M_r \sim 55,000$), high molecular weight-associated proteins [$M_r \sim 270,000$ (refs 4, 5)] and several lower molecular weight components, among them the τ factor bands⁶ of $M_r \sim 60,000$ – $70,000$ (not shown). Analytical ultracentrifugation of the cold protein solutions yields two main components at 6S and 30S. 6S is the value of the α - β heterodimer of tubulin which constitutes the basic building block of microtubules. 30S is a ring-like structure made up of tubulin and MAPs⁵. The rings may be observed by negative stain electron microscopy (Fig. 4, left). Their apparent diameters vary from 320 to 430 Å. The polymerized microtubules are shown in Fig. 4, right. From the X-ray patterns of oriented gels⁷ their mean diameter was determined previously to be ~ 220 Å.

The process of microtubule assembly is frequently monitored by UV light scattering or turbidity. The increase in the scattered intensity following the temperature jump is usually interpreted as microtubule growth, even though any assembly of tubulin into polymorphic forms produces very similar traces. Thus light scattering by itself is a nonspecific measure of overall aggregation. If the experiment is performed in the conditions of Fig. 1, we observe a decrease in turbidity before the onset of polymerization (not shown). This shows the correspondence between the time dependence of turbidity and the central X-ray scattering and emphasizes the additional information contained in the complete X-ray traces.

Interpretation of scattering curves

The changes in intensity and position of the X-ray scattering peaks could be used simply as indicators of the kinetics of assembly. However, the information above allows a structural interpretation using the theory of small-angle scattering⁸. In the approximation that all particles in solution scatter independently, one can express the intensity as

$$I(h, t) \propto \sum_k x_k(t) p_k(t) i_k(h)$$

where $h = 4\pi \sin \theta / \lambda$. The sum is taken over all aggregates k in solution. $x_k(t)$ is the fraction of subunits (tubulin dimers) incorporated into aggregate k ; mass conservation can be

expressed as $\sum_k x_k(t) = 1$. The degree of polymerization $p_k(t)$ expresses the number of subunits per aggregate k , and $i_k(h)$ is the characteristic scattering function of aggregate k normalized to unity at $h = 0$. It contains the structural information. At very small angles $i_k(h)$ approaches 1 so that $I(h, t)$ tends to $\sum_k x_k(t) p_k(t)$. Therefore the central scatter measures the overall aggregation of the system and corresponds to the intensity of scattered UV light. At larger angles the X-ray scattering reflects the overall shape of the aggregates, giving rise to a series of maxima.

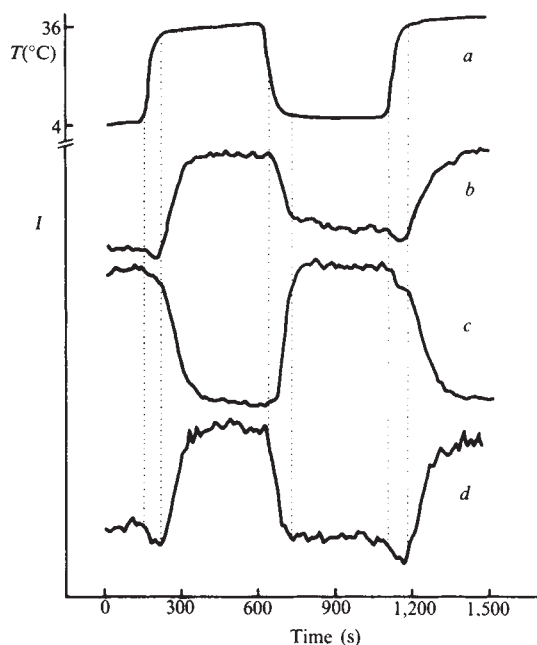


Fig. 3 Time dependence of selected regions of the scattering curves in a temperature jump experiment. *a*, Temperature, measured directly in the protein solution by means of a thermocouple. *b*, Channels 1–5 (central scatter). After the T-jump there is a lag of 45–60 s during which the intensity decreases (undershoot), followed by a sharp rise of half time 100 s. After the reverse T-jump to $4\text{ }^{\circ}\text{C}$ there is a lag of 30–40 s during which the intensity stays constant. This is followed by a biphasic decrease of half times 50 s and 180 s. Depolymerization sets in when the temperature in the solution is about $15\text{ }^{\circ}\text{C}$. *c*, Channels 11–21 (first subsidiary maximum of the cold solution). The intensity drops steadily in a biphasic manner following the T-jump to $36\text{ }^{\circ}\text{C}$. The first phase corresponds to the undershoot of curve *b*, the second phase to the rise of *b*. *d*, Channels 22–32 (first subsidiary maximum of the warm solution). The increase in intensity is preceded by an undershoot whose minimum is reached 30–45 s after the one shown by the central scatter. Similarly, the increase in intensity lags behind that of the central scatter. Following the reverse T-jump the intensity decrease is monophasic and coincident with the first phase of the decrease of the central scatter (*b*).

By subtracting the first intermediate scattering pattern, obtained during the undershoot, from the initial cold one (Fig. 2), one obtains the trace of Fig. 5. The comparison with the model calculation shows that it corresponds to the scattering of rings in solution. In this experiment the best fit of the mean diameter is 375 Å. This value is somewhat larger than the mean diameters deduced from published figures based on electron micrographs (~ 350 Å, see ref. 9). The small discrepancy is probably due to shrinkage on the EM grid. The fact that the difference scattering curves are dominated by the scattering from rings proves that they are dissolved as the temperature is raised, before the formation of microtubules. Within the limits of the instrumental sensitivity the dissolution of rings seems to

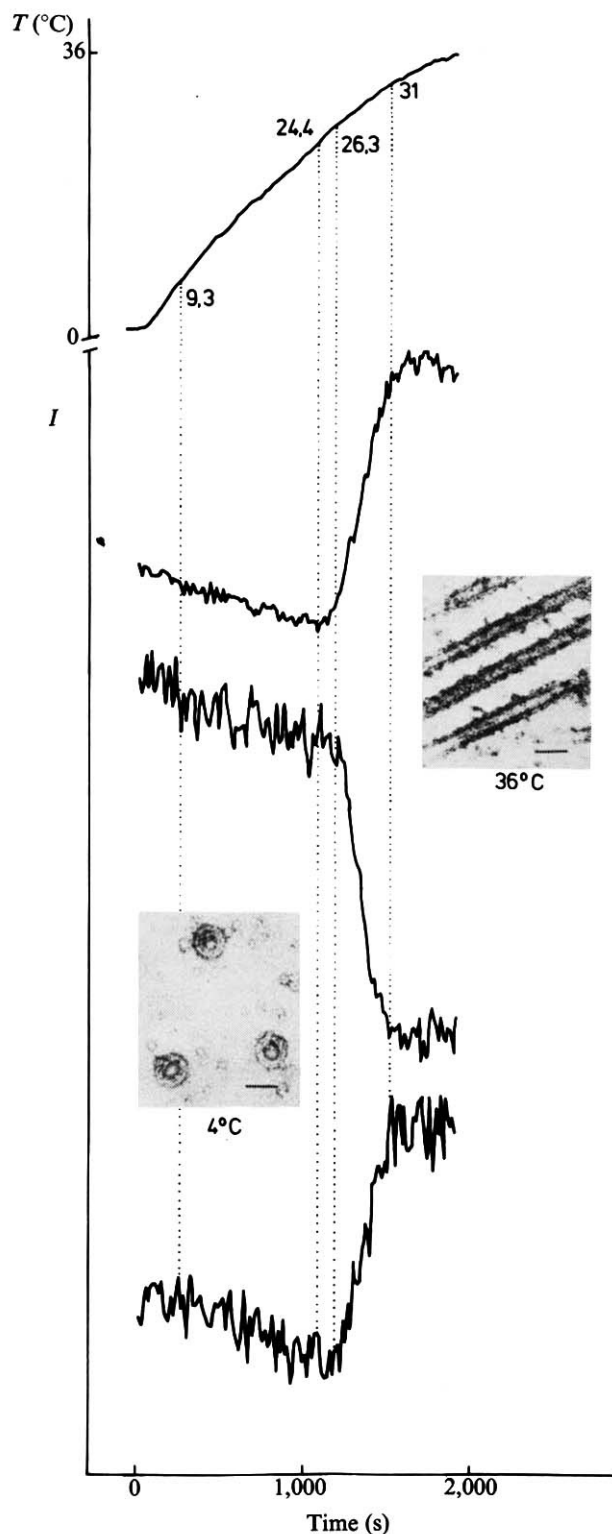


Fig. 4 Microtubule polymerization at a low heating rate. *a*, Temperature. *b-d*, Intensities in the regions corresponding to Fig. 3*b-d*. The central scatter decreases monotonously until 24.4 °C and then rises again (*b*). The region of the first microtubule peak behaves similarly, with the transition temperature displaced towards 26.3 °C (*d*). The region of the first ring peak/first microtubule node decreases monotonously. There is a slight change in rates around 9.3 °C. The second main phase sets in at 26.3 °C. At this point the contribution of rings to the X-ray pattern is below detectability (*c*). Polymerization of microtubules is complete around 31 °C. Insets, Electron images of negatively stained rings (left) and thin sections of microtubules (right). Bar = 500 Å. The cold solution shows single and double rings whose diameters vary between 320 and 430 Å. The warm solution contains microtubules of outer diameters around 250 Å.

be complete by ~60 s after the T-jump (Fig. 2*c*). The processes of ring break down and microtubule formation may be separated particularly well in conditions where the lag time is increased, for instance by raising the temperature slowly (Fig. 4). The transition temperature of about 25 °C coincides with a change in the thermodynamic parameters of the protein¹⁰.

Removal of the contribution of rings from the cold pattern leaves a scattering curve which may be interpreted in terms of scattering from a mixture of dimers and other species smaller than rings. These could be MAPs and/or other tubulin oligomers whose structure is under study.

The scattering pattern of the high temperature state is essentially that of microtubules of mean diameters ~260 Å. We note that their sizes are greater than determined previously in different experimental conditions⁷. This is probably accounted for by the larger contribution of MAPs attached to the outsides of the microtubules (details elsewhere).

Summarizing the above arguments we may interpret the intensity changes qualitatively as follows: The central scatter ($h \approx 0.015 \text{ \AA}^{-1}$) indicates the average degree of polymerization.

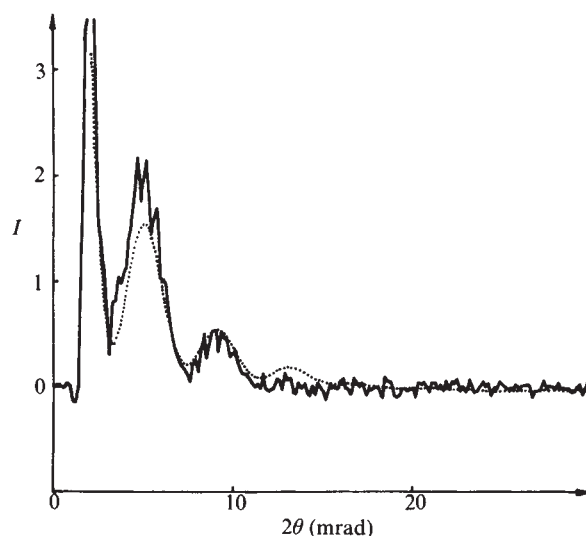


Fig. 5 Difference curve, obtained by subtracting the intensity at 45 s after the T-jump of Fig. 1 from the intensity of the initial cold solution. This plot is representative of the structures that are dissolved. The dotted trace is the calculated small-angle scattering of rings of mean diameter 375 Å, radial width 70 Å, and axial height 50 Å. The positions of the subsidiary maxima are essentially determined by the mean diameter while the width and height modifies mainly the relative amplitudes.

When rings break down its intensity (and that of the rest of the pattern) decreases. It increases again when microtubules are formed (Figs 3*b*, 4*b*). The region around $h \approx 0.02 \text{ \AA}^{-1}$ corresponds to the first subsidiary ring peak. It decreases both absolutely and relative to the rest of the pattern when rings break down. The same region is also near the first subsidiary microtubule minimum. Therefore the intensity decreases even further when microtubules appear (Figs 3*c*, 4*c*). Around $h \approx 0.03 \text{ \AA}^{-1}$ we observe the first subsidiary microtubule peak. The intensity decreases during the breakdown of rings and rises sharply with microtubule polymerization. These rules serve as a guide to assess the system's behaviour quickly (for example in Fig. 1), provided that they are backed up by a detailed comparison of the total scattering curves with model calculations such as Fig. 5. The delay between the intensity increases of the central scatter and the microtubule peak (Figs 3*b*, *d* and 4*b*, *d*) suggests that some transient structure is formed

around 25 °C between the breakdown of rings and polymerization of microtubules (compare with Fig. 2*b, c*). This could correspond to the nucleating centre in the proper sense.

Implications for models of microtubule assembly

Published reports on microtubule polymerization contain a great variety of assembly models (reviewed in ref. 9). Although some of their differences may be due to preparative procedures we suspect that ambiguities inherent in the experimental data play an equally important role. The two main techniques used have been UV light scattering or turbidity and electron microscopy. Turbidity measures only overall polymerization without distinguishing between microtubules and other tubulin aggregates, let alone possible changes of effective diameters due to variations in protofilament number and/or MAP content. Electron microscopy records the structures distorted by drying and staining, it is biased towards large and visible aggregates and provides only snapshot views of the kinetic process. Thus the

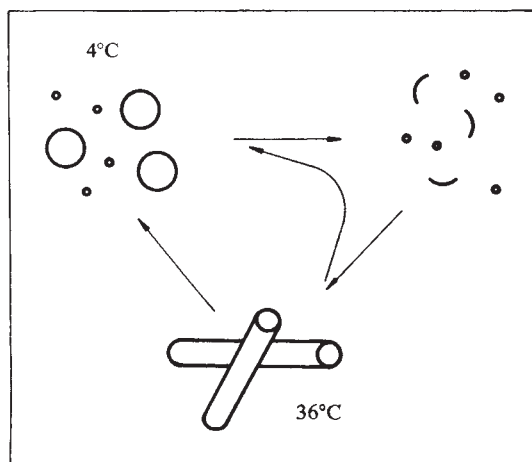


Fig. 6 Possible pathway of tubulin assembly. The initial cold solution contains tubulin dimers, rings, and other entities that are smaller than rings. After the T-jump to 36 °C the rings break apart into fragments which may be involved in the nucleation of assembly. The warm solution consists mainly of microtubules. Depolymerization may proceed either directly to the initial cold state, or via small oligomers.

technique of time-resolved X-ray diffraction helps to bridge the gap between the two conventional approaches as it can be applied to solutions and yet contains structural information on aggregates of all sizes.

The assembly models can be placed in three categories depending on the roles assigned to rings. Some assume that opened-up rings are responsible for nucleation and, together with tubulin subunits, contribute to the elongation of microtubules, possibly through intermediates such as sheets or twisted ribbons¹¹. Others see a role of rings only in nucleation whereas elongation proceeds by end-on addition of 6S tubulin

subunits¹². Some authors have questioned the role of rings altogether as conditions of microtubule assembly can be found in the absence of rings¹³. There seems to be agreement, however, that brain microtubule assembly is most efficient when starting from a ring-containing solution.

The results presented here favour the view that rings do not directly participate in microtubule nucleation or growth as they tend to break down before microtubules appear. By implication, other intermediates seen in the electron microscope such as sheets or ribbons might also be formed from ring fragments and not by association of opened-up rings. These findings support those of Weisenberg¹⁴ who demonstrated the breakdown of the 30S aggregate into smaller units before microtubule assembly.

During disassembly we observe a smooth transition from the scattering pattern of microtubules to that of rings. This could mean that the rings are formed directly as breakdown products of microtubules. Alternatively, if individual tubulin subunits or small oligomers dissociate from the microtubules they must rapidly reassociate into rings. Whatever the mechanism, the nearly parallel decrease of the central scatter and microtubule maxima (Fig. 3*b, d*) suggests that the tubules are largely depolymerized from their ends. The results are summarized in Fig. 6.

Main conclusions and future outlook

The main points to emerge from this study are the following: (1) A cold solution of microtubule protein mainly consists of rings, dimers and other entities made up of MAPs and/or tubulin dimers. (2) When the temperature is raised, either by a T-jump or gradually, rings dissolve into structures of lower degree of polymerization. (3) Within the sensitivity of the method the dissolution of rings is complete before the formation of microtubules. (4) There are at least two intermediate states which differ from both the initial cold and the final warm solution. The first corresponds to the dissolution of rings, the second to the appearance of a new structure just before microtubules begin to form. (5) It is possible to extract accurate sizes for rings and microtubules in solution. (6) Depolymerization is not the exact inverse of polymerization. (7) The cycles of assembly and disassembly can be repeated until the depletion of GTP. These conclusions apply for solutions of microtubule protein prepared in the absence of glycerol, with concentrations around 10–20 mg ml⁻¹.

The aim of this experiment, to establish the use of this technique for monitoring the kinetic and structural transitions occurring during the assembly of macromolecules in solution, is fulfilled for microtubule assembly *in vitro*. The structures formed and their kinetics of polymerization do not seem to suffer significantly from radiation damage during the experiment. The extension of the technique to stopped-flow experiments at constant temperature may have a more direct relationship with physiological conditions. This might enable us to investigate the action of factors promoting assembly, especially that of microtubule associated proteins.

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Protein-linked RNA of poliovirus is competent to form an initiation complex of translation *in vitro*

Fred Golini*, Bert L. Semler, Andrew J. Dorner & Eckard Wimmer†

Department of Microbiology, State University of New York at Stony Brook, Stony Brook, New York 11794

*Present address: Department of Cellular Biology, Scripps Clinic and Research Foundation, 10666 North Torrey Pines Road, La Jolla, California 92037

†To whom correspondence should be addressed

Poliovirus RNA that had been labelled with ^{125}I in the 5'-terminal protein (VPg) was found competent to form an initiation complex of translation in a cell-free reticulocyte lysate. In conditions of ribosome binding, no cleavage occurred between VPg and RNA. We conclude that removal of VPg from poliovirus RNA is not a prerequisite for this RNA to initiate translation in vitro.

BOTH *in vivo* and in cell-free extracts, picornavirus RNA is a highly efficient messenger capable of initiating translation at a much faster rate than most host cell mRNAs^{1,2}. Evidence from *in vitro* experiments indicates that picornavirus RNA is preferentially translated over cellular mRNAs by virtue of its ability to out-compete host cell mRNA for limiting amounts of a messenger discriminatory initiation factor³. The structural feature of picornavirus RNA responsible for its superior translation is not known. Unlike most cellular and viral messengers which require a 5'-terminal 7mG-containing 'capping group' for efficient translation, picornavirus RNA is not capped. Instead, the 5' terminus of virion RNA is linked to the small virus-encoded protein, VPg (refs 4–8). In contrast, polio RNA isolated from polysomes lacks VPg and is terminated with a 5' monophosphate⁹.

Picornavirus RNA is accurately translated in the messenger-dependent reticulocyte lysate system. Processing of precursor polypeptides occurs to produce proteins which correspond to those seen *in vivo*¹⁰. Proteinase K treatment of polio virion

RNA has been shown to degrade VPg proteolytically, leaving a few amino acid residues linked to the intact RNA molecule⁶. This treatment does not destroy the infectivity of the RNA¹¹. Preliminary experiments showed that amino acid incorporation in the reticulocyte lysate was stimulated to the same extent and with identical kinetics by untreated and proteinase K-treated polio RNA. Furthermore, the proteins synthesized in response to both RNAs were indistinguishable when analysed by SDS-polyacrylamide gel electrophoresis (see below). Essentially the same results have been reported for mengovirus RNA treated with proteinase¹².

These results can be interpreted in two ways. In the first case, it may be that the presence or absence of VPg has no effect on the translation of picornavirus RNA. Alternatively, the cell-free system may be able to cleave VPg from RNA so efficiently that no apparent difference is seen in incubations programmed with the two messengers. An activity capable of removing VPg from RNA in extracts prepared from a variety of cells has been described¹³. However, as only a small proportion of the RNA

Fig. 1 Binding of polio RNA to reticulocyte ribosomes. Poliovirus ^{32}P -RNA and ^{125}I -VPg-RNA (from type 1, Mahoney) were prepared as described previously^{9,16}. The messenger-dependent reticulocyte lysate was prepared according to the method of Pelham and Jackson²⁰. Incubation mixtures (100 μl) were assembled at 4°C, containing nuclease-treated reticulocyte lysate (50 μl), creatine phosphokinase (10 μg), 10 mM phosphocreatine, 20 amino acids (0.1 mM each), 20 mM Tris-HCl, pH 8.2, 3 mM $\text{Mg}(\text{OAc})_2$, 0.13 M KOAc, 2 mM dithiothreitol, reticulocyte ribosomal RNA (1 μg), 0.25 mM NAD, 0.1 mM sparsomycin, and 250 guinea pig L.D. per ml diphtheria toxin. After preincubation at 30°C for 4 min, 0.1 μg labelled polio RNA was added and incubation continued for 4 min. An equal volume of 1% sodium deoxycholate, 1% Triton X-100 was added and 1 min later the incubation was terminated by chilling to 4°C. Initiation complexes were resolved by sedimentation at 4°C on 15–30% w/w sucrose gradients in buffer F (25 mM KCl, 10 mM NaCl, 1.5 mM $\text{Mg}(\text{OAc})_2$, 10 mM Tris-HCl, pH 7.5 (ref. 14), supplemented with 0.5% sodium deoxycholate and 0.5% Triton X-100. Experiments containing polio ^{32}P -RNA (a, c) were centrifuged for 10 h at 29,000 r.p.m. in the Spinco SW41 rotor. For b, the incubation mixture was scaled up fivefold and programmed with ^{125}I -VPg-polio RNA. Centrifugation was for 10 h at 27,000 r.p.m. in the SW27 rotor. Radioactivity in sucrose gradient fractions was determined in a liquid scintillation counter after addition of one half-volume of H_2O and nine volumes of Biofluor (NEN). ○, Standard conditions; ●, preincubation mixture contained either 5 μg unlabelled polio RNA (a) or 10^{-6}M ATA (b, c). The S value of the ribosome complex was determined by incubating the lysate with ^{35}S -tRNA^{met} and globin mRNA or poliovirus RNA in the same conditions as described for a, and sedimenting the initiation complex through a sucrose gradient²¹.

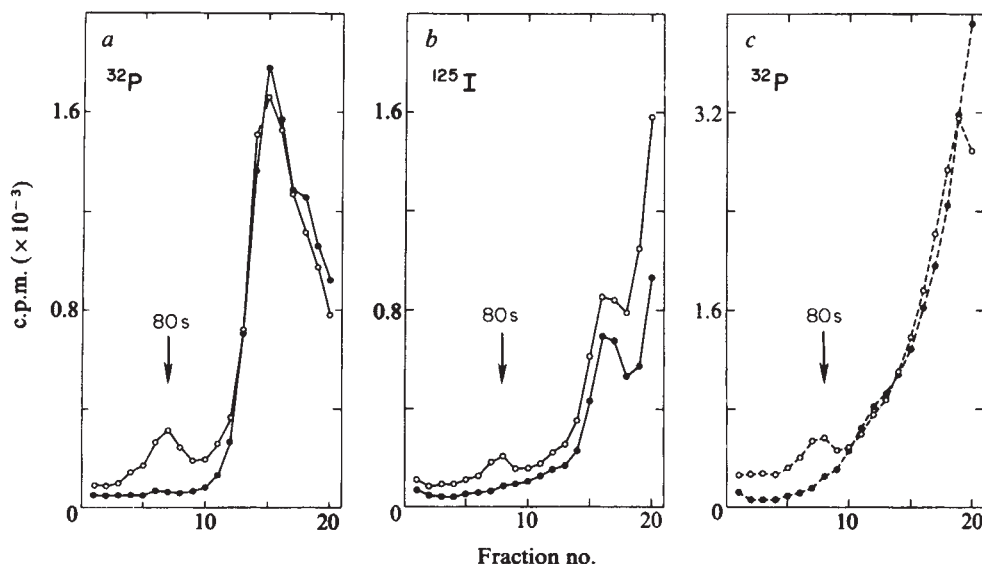
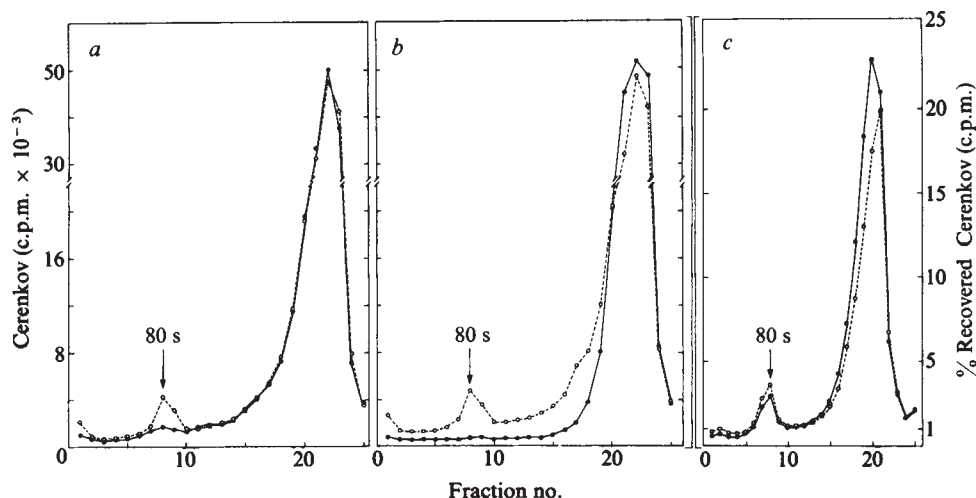


Fig. 2 Binding of polio RNA to reticulocyte ribosomes. Incubation mixtures of the messenger-dependent reticulocyte lysate were prepared as described in Fig. 1 legend. Standard condition reactions were programmed with virion ^{32}P -RNA at a final concentration of $2\ \mu\text{g ml}^{-1}$. Centrifugation was for 16 h at 21,000 r.p.m. in the SW27 on gradients as described in Fig. 1 legend. Radioactivity in gradient fractions was determined directly by Cerenkov counting. Proteinase K digestion of polio ^{32}P -RNA was done at 37°C for 1 h¹¹. The reaction mixture contained 10 mM Tris, pH 7.6, 100 mM NaCl, 1 mM EDTA, 0.5% SDS, proteinase K ($500\ \mu\text{g ml}^{-1}$) and polio RNA ($300\ \mu\text{g ml}^{-1}$). The mixture was phenol/chloroform extracted after the incubation and the RNA ethanol precipitated before use in the reticulocyte lysate. ○, Standard conditions; ●, preincubation mix contained pactamycin at a final concentration of: a, $4 \times 10^{-6}\ \text{M}$; b, globin mRNA at a final concentration of $400\ \mu\text{g ml}^{-1}$; c, the reaction was programmed with proteinase K-treated ^{32}P -RNA at a final concentration of $2\ \mu\text{g ml}^{-1}$.



added to *in vitro* cell-free systems is actually translated, the fate of VPg on those molecules which function as messenger was not ascertained. The approach we used to resolve this question was to isolate initiation complexes of ribosomes bound to polio RNA and then determine if VPg was retained on this fraction.

Characterization of initiation complex

Reticulocyte lysates supplemented with sparsomycin and diphtheria toxin allow the binding of ribosomes to mRNA and synthesis of the first peptide bond but block further elongation, permitting isolation of initiation complexes by sucrose gradient centrifugation as shown in Fig. 1 (ref. 14). Due to the large sedimentation coefficient of picornavirus RNA and its nonspecific association with cytoplasmic proteins, separation of ribosome-bound and unbound RNA is difficult. This problem can be overcome by the addition of deoxycholate and Triton X-100 to the incubation and gradient buffer. In the conditions described in Fig. 1 legend, 10% of the polio ^{32}P -RNA is found associated with ribosomes in a peak sedimenting at approximately 80S. The remaining RNA is recovered as a broad peak at 60S with a pronounced shoulder skewed towards the top of the gradient, an observation suggesting that ribonuclease degradation of the unbound RNA occurs. Several criteria have been used to establish that the more rapidly sedimenting peak in Figs 1 and 2 represents a true initiation complex. In control reactions kept at 0°C , the 80S peak was not seen (data not shown). In Fig. 1a, it can be seen that preincubation with excess nonradioactive polio RNA saturates the capacity of the lysate and prevents subsequent binding of ^{32}P -RNA. The same effect was observed when the lysate was preincubated with nonradioactive globin mRNA (Fig. 2b). Addition of aurin tricarboxylic acid (ATA), an antibiotic which inhibits association of ribosomes with mRNA, or pactamycin, an inhibitor of initiation of translation¹⁵, both prevent the appearance of the faster sedimenting peak (Figs 1c, 2a, respectively).

The initiation complex formed with polio ^{32}P -RNA has been further characterized by treatment with ribonuclease T1 and isolation of the ribosome-protected fragment. Analysis by polyacrylamide gel electrophoresis reveals that one major band about 40 nucleotides long is obtained (arrow, Fig. 3a, lane 2) together with other RNA bands. This band has been reproducibly observed in several independent experiments using intact virion RNA, or, as shown in Fig. 3b, lane 1, using autoradiolysed virion ^{32}P -RNA. The length of the other RNA fragments, however, changes from experiment to experiment (Fig. 3b, lane 1). The major band (arrow) is not generated when virion ^{32}P -RNA is bound to ribosomes, isolated without RNase T1 treatment, and analysed by acrylamide gel electrophoresis (Fig. 3b, lane 2). The major fragment protected by ribosome binding is capable of rebinding to ribosomes if the mixture of RNA fragments is subjected to a second cycle of ribosome binding (Fig. 3a, lane 3). We cannot rule out the possibility that more than one RNA species rebound to ribosomes because the amount of radioactivity in this lane was low.

The VPg moiety of polio genome RNA was specifically labelled with ^{125}I using the Bolton-Hunter reagent¹⁶. The results of ribosome-binding experiments with this RNA are shown in Fig. 1b. Approximately 5% of the labelled RNA is recovered in the initiation complex, showing that VPg is retained on a significant fraction of the polio RNA molecules bound to ribosomes. Formation of the complex is sensitive to ATA. The specific labelling of VPg has been confirmed by releasing ^{125}I -VPg from the initiation complex with RNase T2 and analysing it by gel electrophoresis as published previously¹⁶. The only radioactive product seen is VPg migrating to the same position as control VPg (data not shown). Unlike the results obtained using ^{32}P -RNA, the peak of unbound ^{125}I -VPg-RNA (in the right half of Fig. 1b) is relatively smaller and most of the radioactivity sediments more slowly. The reason for this is not understood.

Table 1 Determination of degree of cleavage of ^{125}I -VPg from ^{125}I -VPg-RNA in various incubations

Time (min)	No inhibitors			+ Sparsomycin + Diphtheria toxin			+ ATA		
	Aqueous phase (c.p.m.)	Organic phase (c.p.m.)	% Aqueous phase	Aqueous phase (c.p.m.)	Organic phase (c.p.m.)	% Aqueous phase	Aqueous phase (c.p.m.)	Organic phase (c.p.m.)	% Aqueous phase
0	3,473	152	96	3,421	154	96	3,181	125	96
10	3,436	177	95	3,372	192	95	3,253	211	94
20	3,333	243	93	3,331	239	93	3,212	244	93
30	3,116	465	87	3,088	565	85	2,929	488	86

Incubation mixtures (500 μl) were prepared as described in Fig. 1 legend with and without the inhibitors as indicated. At the times indicated, aliquots were removed and extracted with an equal volume of phenol/chloroform (1:1). Biofluor (NEN) was added to each of the samples and radioactivity was determined by scintillation counting.

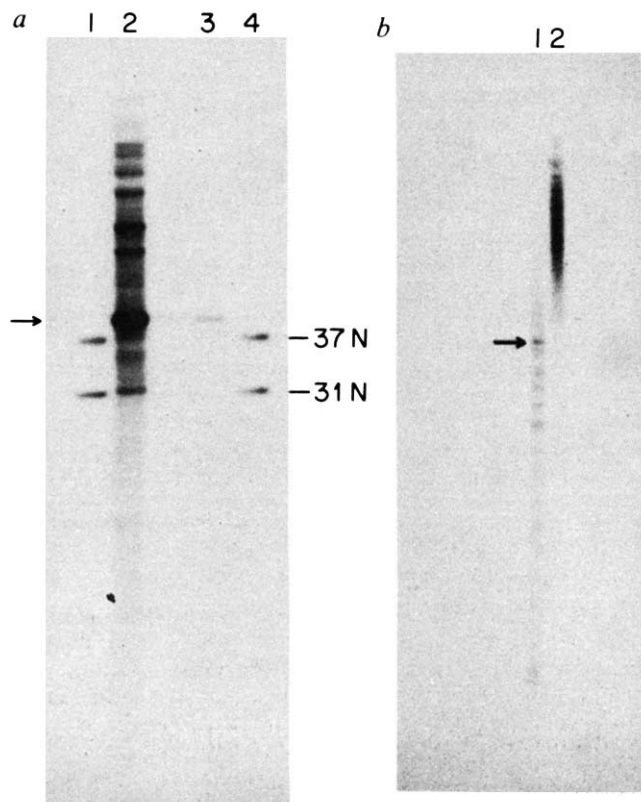


Fig. 3 Acrylamide gel electrophoresis of the ribosome-protected fragment(s) of ^{32}P -RNA from poliovirus. *a*, ^{32}P -RNA from poliovirus was bound to reticulocyte ribosomes as described in Fig. 1 legend. The peak fractions of the sucrose gradient (sedimenting at $\sim 80\text{S}$) were pooled. RNase T1 was added to 20 units ml^{-1} and incubated at 37°C for 10 min. The mixture was then chilled on ice and diluted with an equal volume of ice-cold buffer F, containing 0.5% deoxycholate and 0.5% Triton X-100. The sample was pelleted through a 5-ml cushion of 15% sucrose (in buffer F) by centrifugation in the SW27 rotor at 27K r.p.m. (4°C) for 12–14 h. The pellet was resuspended in 0.5 ml of column buffer (10 mM Tris-HCl, pH 7.5, 1 mM EDTA, 7 M urea) containing 0.1% SDS and extracted twice with phenol/chloroform (1:1). The aqueous phase was then applied to a small DEAE-cellulose column (0.5 $\times$ 4 cm) that had been equilibrated with column buffer. The column was washed with the same buffer containing an additional 0.15 M NaCl (5–10 ml). The presumptive ribosome-protected fragment(s) was eluted with column buffer containing 0.5 M NaCl. The solution containing the fragment(s) was divided. To one half, carrier (either tRNA to 20 $\mu\text{g ml}^{-1}$ or purified oyster glycogen to 200 $\mu\text{g ml}^{-1}$) was added and the sample was alcohol precipitated. This RNA was then analysed by gel electrophoresis on a 20% acrylamide slab gel containing 7 M urea (lane 2). Fragment(s) in the other half were subjected to a second cycle of the same binding and isolation experiments, and analysed by gel electrophoresis (lane 3). Lanes 1 and 4 are marker T1-oligonucleotides 1 and 3 from ^{32}P -RNA of poliovirus, 37 and 31 nucleotides long²². N, Nucleotides. *b*, Samples were prepared as described in *a*, except that autoradiolysed virion ^{32}P -RNA was used in the binding experiments and the DEAE cellulose column step was omitted. Lane 1, ribosome-protected fragments corresponding to lane 2 in *a*; lane 2, ribosome-protected RNA isolated without the RNase T1 treatment step.

Retention of VPg on virion RNA after initiation

In view of the activity in uninfected cell extracts that cleaves the linkage between polio RNA and VPg¹³, we considered it possible that, by freezing the ribosome on the initiation site, we might prevent the removal of VPg subsequent to the initiation step. Three protein synthesis incubations were prepared containing ^{125}I -VPg-RNA and supplemented with no inhibitor, sparsomycin and diphtheria toxin, or ATA. Aliquots were removed at various times and the unlinking of VPg determined by the phenol extraction assay¹³. Similar results were obtained in the case of all three incubations: little cleavage of VPg from RNA was detected (Table 1). Thus, the retention of VPg on virion RNA reported here is not a consequence of blocking

translation at the initiation step. The reason for the apparent discrepancies between our results of release of VPg from RNA and those of Ambros *et al.*¹³ may be due to differences in the method of preparation of the reticulocyte extracts and conditions of incubation. A wash of HeLa cell nuclei¹³, on the other hand, did remove 90% of VPg from $[\text{Tyr-}^3\text{H}, ^{32}\text{P}]\text{VPg-RNA}$ using the same phenol extraction assay (P. G. Rothberg and E.W., unpublished).

Role of VPg

If the presence of VPg on virion RNA did not prevent ribosome binding, it seemed possible that it might have at least lowered the efficiency of initiation complex formation. Therefore, we compared ribosome binding using VPg-RNA and virion RNA, the VPg of which was degraded proteolytically. As can be seen in Fig. 2c, both RNAs form the initiation complex to the same extent. Similarly, translation of VPg-RNA and proteinase K-treated RNA in the reticulocyte lysate leads to the same translation on products with similar yield (Fig. 4).

The role of VPg is not known but the appearance of protein-linked RNA during replication is a general feature of all

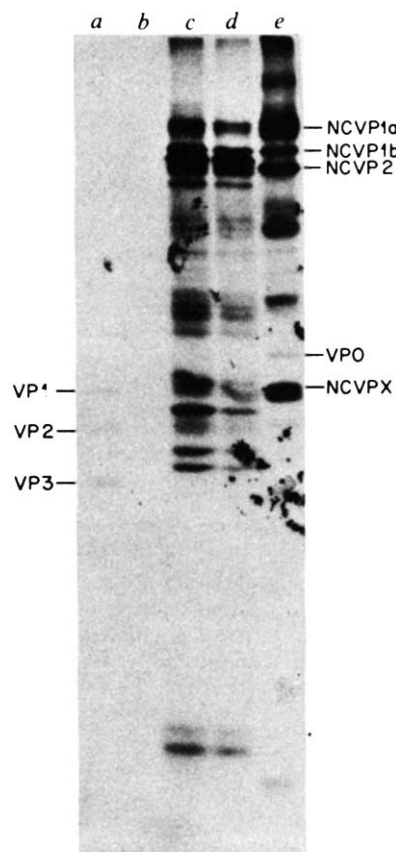


Fig. 4 Acrylamide gel electrophoresis of polio-specific proteins synthesized in the reticulocyte lysate. The messenger-dependent reticulocyte lysate was prepared according to the method of Pelham and Jackson²⁰. Reaction mixtures (25 μl) contained 0.5 vol reticulocyte lysate, 20 mM Tris, pH 8.0, 2 mM dithiothreitol, 100 mM KOAc, 1 mM $\text{Mg}(\text{OAc})_2$, 0.5 mM spermidine, 80 μM amino acids except methionine, 50 $\mu\text{g ml}^{-1}$ tRNA, 20 mM creatine phosphate, 100 $\mu\text{g ml}^{-1}$ phosphocreatine kinase, 0.01 $\mu\text{g ml}^{-1}$ rRNA, 60 $\mu\text{g ml}^{-1}$ polio RNA and 400 $\mu\text{Ci ml}^{-1}$ ^{35}S -methionine (Amersham). Incubation was for 2 h at 30°C . Aliquots (1 μl) of the reaction mixture were electrophoresed on 12.5% acrylamide gels containing 0.5% SDS according to the method of Laemmli²³. The gel was subjected to fluorography according to the method of Bonner and Lasky²⁴ and exposed at -70°C . Proteinase K-treated RNA was prepared as described in Fig. 2 legend. Lane *a*, ^{35}S -methionine-labelled capsid proteins isolated from purified virions; lane *b*, without added mRNA; lane *c*, polio virion RNA; lane *d*, proteinase K-treated polio virion RNA; lane *e*, *in vivo* lysate from poliovirus-infected HeLa cells labelled with ^{35}S -methionine. NCVp1a, NCVp1b, NCVp2, VP0 and NCVpX are polio-specific non-copied proteins.

picornaviruses examined¹⁷. Based on the observations that polio mRNA is infectious and that proteinase K-treated and untreated virion RNAs have equal specific infectivities in transfection assays, it seems that VPg is not essential for early events in replication^{6,11}. The identification of VGp on nascent RNA molecules in molar yield implies that it is either a primer for RNA synthesis or is added to all growing chains very soon after initiation has begun^{7,18}.

We have suggested previously that cleavage of VPg from VPg-RNA may be a prerequisite for this RNA to function as mRNA^{7,11}. Our data presented here argue against this hypothesis.

In view of the conclusion reported here that VPg does not interfere with translation *in vitro*, the cleavage of VPg from polysomal RNA *in vivo* is noteworthy. Polio mRNA is not packaged into virions¹⁹. It is tempting to speculate that retention

of VPg may tag those RNA molecules which are to be encapsidated, and thus regulates whether newly synthesized RNA becomes mRNA or enters the pool of progeny genome RNA molecules^{7,11,17}. Proof of such a function for VPg clearly awaits further experimental evidence.

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Functional and non-functional joining in immunoglobulin light chain genes of a mouse myeloma

Werner Altenburger*, Michael Steinmetz† & Hans G. Zachau

Institute für Physiologische Chemie, Physikalische Biochemie und Zellbiologie der Universität München, FRG

Leader, variable (V) and joining (J) gene segments, and adjacent regions of two rearranged alleles of the same κ -chain producing mouse myeloma, comprising approximately 3,200 base pairs, have been sequenced. Sequence comparisons are reported. V-J joining in one of the alleles leads to a reading frame with a stop codon within the J-gene segment. Allelic exclusion is apparently realized in this tumour through the formation of such a non-functional allele.

In germ-line DNA the information for leader, variable, joining and constant regions of immunoglobulin light chains is located on four separate gene segments called L, V, J and C. In differentiation to an immunocompetent cell, a V- and a J-gene segment are joined by a site-specific recombination process^{1,2}. Generally only one type of immunoglobulin is expressed originating from one of the two alleles of the cell^{3,4}; this has been termed allelic exclusion. In 15 of 30 mouse myelomas examined allelic exclusion seems to result simply from the fact that only one allele has undergone the V-gene rearrangement whereas the second allele remains in its germ-line configuration⁵. In other myelomas more than one allele was found to be rearranged⁵⁻⁹. In some, protein and/or genomic analyses clearly showed that one of the translocations was grossly aberrant¹⁰⁻¹³. However, in other myelomas it was not apparent that one translocation was aberrant or it was even shown that both

translocations occurred in the J-gene cluster¹⁴. Assuming that allelic exclusion operates also in those myelomas one has to search for other reasons either in the translocation step or on the levels of transcription or translation.

Two rearranged κ light chain genes in one mouse myeloma have been previously described⁸. The cloning and restriction mapping of the two rearranged alleles on 14-kilobase⁸ and 20-kilobase *EcoRI* fragments¹⁴, respectively, were also reported. We used the myeloma from which the κ chain cDNA clone k38 was derived by Mach *et al.*¹⁵ and which was thought to be identical to MOPC173. However, the sequence analysis described here shows that the V genes on k38 and on the two rearranged *EcoRI* fragments of the myeloma do not correspond to the amino acid sequences of MOPC173 (ref. 16) or other mouse myelomas¹⁷. Apparently a myeloma other than MOPC173 had been used for the isolation of the mRNA and the subsequent preparation of clone k38 (ref. 15). The same conclusion was reached in independent sequence work on the 14-kilobase fragment and the k38 clone by Fiori, Gorski and Mach (manuscript in preparation). To avoid introducing a new number into the notation before the myeloma is further

* Present address: Institut für Molekularbiologie II der Universität Zürich, Hönggerberg, 8093 Zürich, Switzerland.

† Present address: California Institute of Technology, Division of Biology, Pasadena, California 91125.

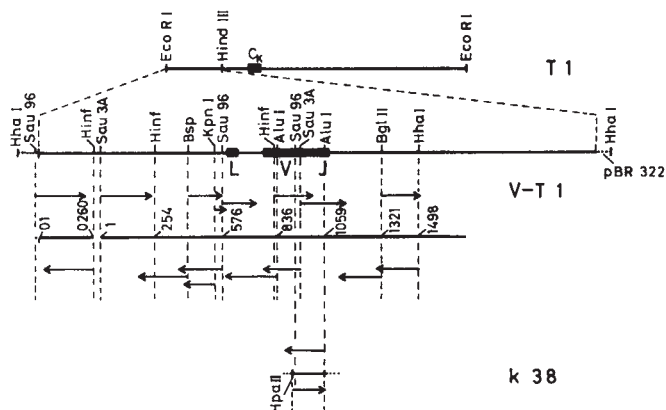


Fig. 1 Localization of the pBR322 subclone V-T1 on the 14-kilobase *EcoRI* fragment T1¹⁴ and partial restriction map of V-T1. Digestions were as previously described¹⁴. DNA fragments were isolated by *HhaI* (or *HpaII*) cleavage and separated from the small pBR322 fragments by isokinetic³¹ sucrose gradient centrifugation (1 M NaCl, 20 mM Tris-HCl, 10 mM EDTA, pH 8.0; *C* = 5%, particle density 1.89, rotor SW 27.1 for 40 h, 25,000 r.p.m., 20 °C, 1.5–2.0 mg DNA per tube). The fractions (taken from bottom to top) were tested on 1% horizontal agarose gels, pooled, precipitated with ethanol, and washed twice with 80% ethanol. 0.15–0.20 mg of the *HhaI* (or *HpaII*) fragments (isolated in about 90% yield) were cleaved by one of the nucleases as indicated by dotted lines. Phosphatase treatment (67 °C) and the further steps of sample preparation were as in refs 18 and 32. Labelled fragments were purified by a DE52 step before chemical modification. All 90 °C steps were in siliconized capillaries. Direction and extent of the sequencing are indicated by the arrows. The sequencing strategy of the cDNA clone k38 (see text) is indicated in the bottom line.

characterized we called it tumour T (refs 14, 18, 19). Accordingly the 14- and 20-kilobase fragments are designated T1 and T2, respectively. A 5.3-kilobase *Eco*RI fragment called T3 which was cloned from the same tumour¹⁸ is possibly related to the translocation of immunoglobulin gene segments.

Functional join of V-gene segment to J5-gene segment in one allele of tumour T

Our main interest here was to sequence the V gene segments of the two rearranged alleles and the adjacent L and J regions. We decided to extend the sequencing work both upstream and downstream of these regions for the following reasons. The upstream regions are interesting with respect to possible regulatory sequences and will be even more so as soon as comparison with analogous sequences of other immunoglobulin genes becomes possible. The region downstream of the J segments which extends into the intervening sequence (intron) between J and C_K gene segments is interesting with respect to a search for sites of possible aberrant recombinations¹¹⁻¹³.

As can be seen in Fig. 1, the L-, V- and J-gene sequences of the allele which is found on fragment T1 are located on the previously described subclone V-T1 (ref. 14). 1,800 base pairs have been sequenced (Fig. 2). As a starting point for orientation within the sequence we turn to nucleotides 1,029–1,067 which are identical to those of the J5 gene segment as it was sequenced in germ-line DNA^{20,21} (designation as in ref. 20). The junction is within or to the right of the codon CCG, at positions 1,026–1,028. To check whether a V-gene segment is located upstream of the J5-gene segment, the sequence was examined for the presence of codons for the so-called invariant amino acids¹⁷. All characteristic amino acids were found in the expected positions (6, 16, 23, 26, 35, 44, 57, 59, 61, 62, 63, 64, 65, 73, 75, 86 and 88). The amino acid sequence of the V region as deduced from the nucleotide sequence of fragment T1 is not identical to any of the known¹⁷ sequences of κ light chains. It differs from the published sequences within the 23 amino-terminal amino acids in more than four positions and therefore, by definition²², cannot be assigned to any one of the known subgroups.

The L-gene segment was localized in fragment T1 at nucleotides 571–621 (Fig. 2) on the basis of the characteristic²³ amino acids at positions 1, 11, 12 and 17 in the leader sequence. Note that there is another possible start codon two codons upstream from the one of amino acid 1 of the leader sequence. The L gene is interrupted by an intron of 113 nucleotides with the probable splice signals AGGT at both ends (positions 618–

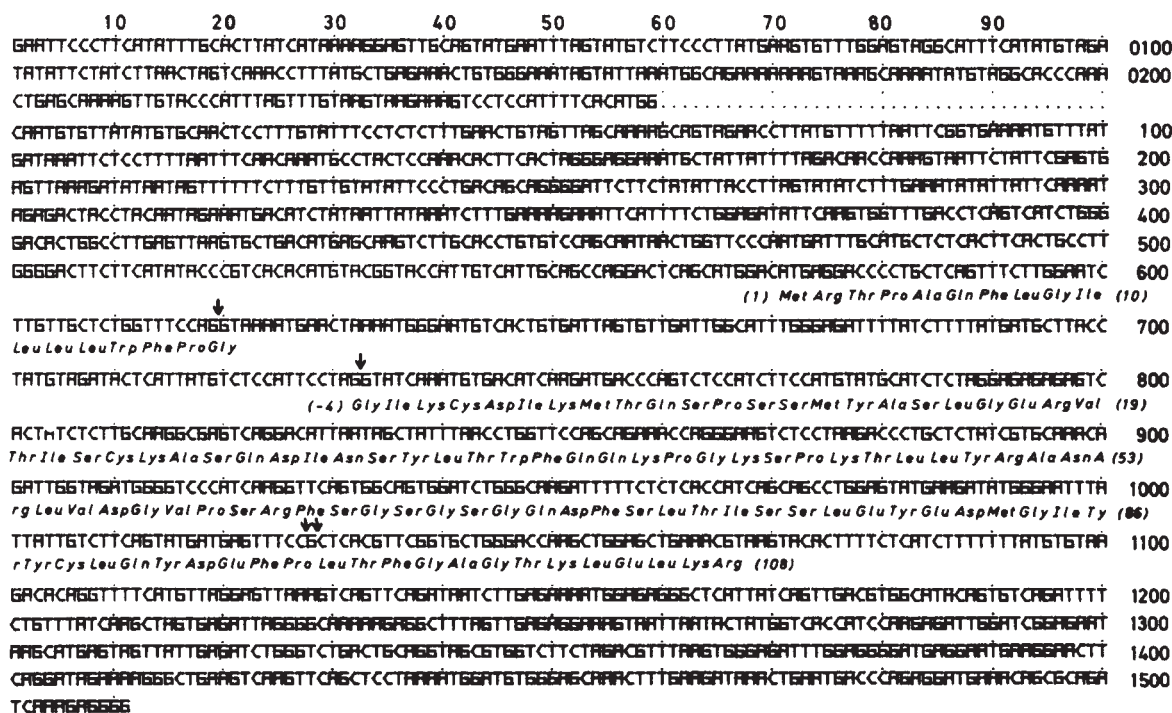


Fig. 2 Nucleotide sequence of the L, V and J regions of fragment T1. Nucleotides are marked 01–0200 and 1–1,500 (interruption of about 40 base pairs between *Hinf* and *Sau3A* sites on the left of Fig. 1), amino acids in L, V, J are (1)–(10) etc. The splice and joining points are marked with vertical arrows. Overlaps with published sequences extend from position 1,028 to 1,356 (ref. 20; differences appear in positions 1,095, 1,119, 1,140, 1,209–1,211 and 1,028–1,118 (difference in position 1,092 with respect to ref. 21)).

J-gene segment. Transcription⁵ and even translation^{10,30} of non-functional immunoglobulin genes have in fact been observed. It has been proposed that the recombination between V- and J-gene segments may occur at slightly different points thereby increasing the diversity of variable regions^{20,21}. This flexible recombination mechanism may also lead to non-functionally recombined V-region genes. It is obviously of greater physiological significance to increase antibody diversity by junctional diversification than to generate a functional gene in every V-J joining step by a more stringent recombination mechanism.

Allelic exclusion may be the result of a multi-step process^{5,30} with V-gene rearrangements taking place until one functional V-J junction has been formed and further rearrangements are

inhibited by an unknown mechanism. The fragment T3 which was found in tumour T and in which 3'-flanking sequences of a V gene and 5'-flanking sequences of a J-gene segment are linked¹⁸ may be the side product of an early translocation step either in the T1 or the T2 allele. Translocation(s) in the T1 allele eventually led to a functional gene while allelic exclusion was realized by a non-functional joining in the T2 allele.

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Phorbol esters and vasopressin stimulate DNA synthesis by a common mechanism

Phillip Dicker & Enrique Rozengurt

Imperial Cancer Research Fund, Lincoln's Inn Fields, London WC2A 3PX, UK

The potent tumour promoter 12-O-tetradecanoylphorbol-13-acetate (TPA) acts synergistically with all known mitogens to induce DNA synthesis in 3T3 cells. In contrast, the neurohypophyseal hormone vasopressin, which is mitogenic for 3T3 cells, fails to synergize with TPA to stimulate DNA synthesis, ornithine decarboxylase activity or 2-deoxyglucose uptake. Thus TPA acts through a pathway which converges with that used by vasopressin, and at least some of its biological effects occur through mechanisms normally utilized by specific hormones.

TUMOUR promoters constitute a family of compounds which, although not carcinogenic by themselves, drastically increase the incidence of tumours when applied repeatedly to animals that have received a sub-threshold dose of a carcinogen¹. Tumour promotion is a phase in the development of many tumours including those that occur in humans^{2,3}. Their mechanism of action is of considerable interest, with attention focusing on their biological effects in various types of cultured cells^{2,4}.

Studies of the cellular actions of 12-O-tetradecanoyl-phorbol-13-acetate (TPA), the most potent tumour promoter of the phorbol ester family², suggested that these compounds regulate a complex array of cellular activities, including mitogenesis, by using receptor-effector systems for a physiologically occurring material^{5,6}. It has been suggested that the biological effects of TPA are mediated by the epidermal growth factor (EGF) receptor-effector system⁷⁻⁹, but although TPA inhibits the specific binding of EGF to the cell, phorbol esters and EGF do not compete directly for the same receptors¹⁰⁻¹². Further, TPA synergistically stimulates DNA synthesis when added in combination with most known mitogens including unfrac-

tionated serum^{4,13,14}, plasma¹⁵, platelet-derived growth factor¹⁵, multiplication-stimulating activity¹⁵, fibroblast-derived growth factor (FDGF)¹⁶, fibroblast growth factor¹⁵, insulin¹⁶ and EGF^{6,10,15,16}, indicating that TPA does not act identically to any of these growth factors. At least two alternative mechanisms of action of TPA can be envisaged. It may act in a fundamentally different way from hormones and growth factors by increasing cellular sensitivity to any growth factor in a non-selective fashion¹⁵, or it may regulate biological activity through a specific hormonal pathway not yet identified^{5,6}.

We have found that the neurohypophyseal nonapeptide arginine vasopressin is a potent mitogen for Swiss 3T3 cells¹⁷. We were led to this finding when testing whether hormones and ionophores able to stimulate ion fluxes¹⁸⁻²⁰ could also regulate cell proliferation. The effect of vasopressin on DNA synthesis is synergistically potentiated by insulin, EGF and FDGF¹⁷. Similarities in the mitogenic properties of vasopressin and TPA prompted us to suggest that these chemically diverse factors stimulate DNA synthesis through an identical mechanism⁶. Based on the evidence presented here, we propose that the actions of TPA and vasopressin converge into a common

pathway to stimulate DNA synthesis, 2-deoxyglucose uptake and ornithine decarboxylase activity in quiescent cultures of mouse Swiss 3T3 cells.

Equivalent mitogenic properties

A series of experiments demonstrated the equivalence of the mitogenic properties of TPA and vasopressin. Addition of saturating levels of either vasopressin or TPA to quiescent cultures of Swiss 3T3 cells stimulated identical increases in DNA synthesis when tested in combination with EGF, insulin, FDGF or low concentrations of serum (1.5–2.5%, Table 1), when DNA synthesis was measured either by ^3H -thymidine (^3H -TdR) incorporation into acid-insoluble material (Table 1) or by the percentage of labelled nuclei after autoradiography (results not shown).

We have found that exposure of cell cultures to vitamin A derivatives such as retinoic acid²¹, or disruption of the microtubular network by colchicine²², enhances the stimulation of DNA synthesis induced by growth factors in quiescent cells. To ascertain whether the mitogenic equivalence of vasopressin and TPA holds under those conditions of increased cellular responsiveness, cultures of quiescent 3T3 cells were exposed to vasopressin plus EGF or TPA plus EGF and to different concentrations of retinoic acid or colchicine. The dose-dependent enhancement of DNA synthesis produced by retinoic acid or by colchicine in the presence of TPA plus EGF is identical to that obtained in parallel cultures exposed to vasopressin plus EGF (Fig. 1). Thus vasopressin and TPA can substitute for each other in stimulating DNA synthesis in Swiss 3T3 cells in the presence

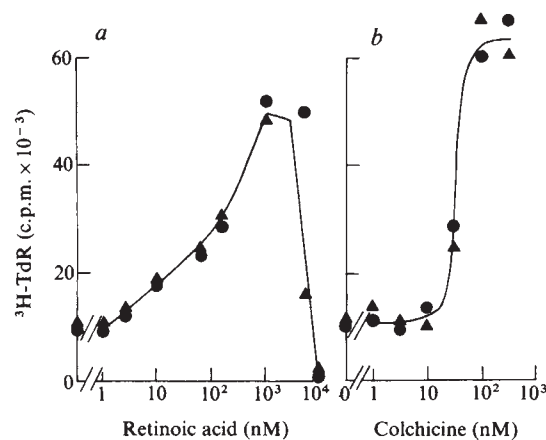


Fig. 1 Enhancement by retinoic acid and colchicine of ^3H -TdR incorporation induced by TPA plus EGF (●) or vasopressin plus EGF (▲) in Swiss 3T3 cells. Confluent and quiescent cultures of 3T3 cells were washed twice with Dulbecco's modified Eagle's medium (DMEM) at 37 °C to remove residual serum, then incubated in 2 ml of a 1:1 mixture of DMEM: Waymouth medium⁴³ containing ^3H -TdR (0.5 $\mu\text{Ci ml}^{-1}$; 1 μM , Amersham) and mitogens as indicated. After 40 h at 37 °C ^3H -TdR incorporation into acid-insoluble pools was assayed as follows. Cultures were washed twice with ice-cold phosphate-buffered saline (0.15 M NaCl in 0.1 M K_2PO_4 buffer, pH 7.4) and acid-soluble radioactivity was removed by a 20-min treatment with 5% trichloroacetic acid (TCA) at 4 °C. Cultures were then washed twice in ethanol, cells were solubilized by a 30-min incubation with 0.1 M NaOH and 2% Na_2CO_3 , and the radioactivity in the acid-insoluble pools was determined. Fetal calf serum (10%) induced incorporation of 155×10^3 and 198×10^3 c.p.m. when retinoic acid and colchicine (Sigma) were involved, respectively. TPA (Consolidated Midland, Brewster, New York), EGF or vasopressin (Sigma) alone induced incorporation of less than 2×10^3 c.p.m. Concentrations of mitogens were 10 ng ml^{-1} , 10 ng ml^{-1} and 100 ng ml^{-1} for vasopressin, EGF and TPA, respectively. All points are the average of duplicate plates. Stock cultures of Swiss 3T3 cells were maintained in DMEM plus 10% fetal calf serum, penicillin (100 U ml^{-1}) and streptomycin (100 $\mu\text{g ml}^{-1}$), in a humidified atmosphere of 10% CO_2 :90% air at 37 °C. Cells were subcultured in 30- or 90-mm Nunc Petri dishes with medium containing 10% fetal calf serum, refed with the same medium after 2 d and used 5 d after the last change of medium. These cells were arrested in G_1 as judged by cytofluorometric analysis (Table 2) and by the fact that less than 1% of cells are autoradiographically labelled after a 40 h exposure to ^3H -TdR. Retinoic acid was dissolved in ethanol. Equivalent concentrations of ethanol were added to all controls and never exceeded 0.5% which was not toxic to our cells. Stock solutions of TPA (10 mg ml^{-1}) were made up in acetone and kept at -20 °C.

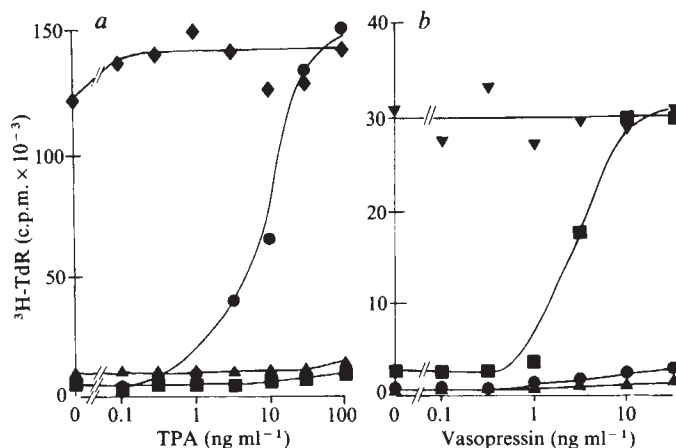


Fig. 2 Absence of synergistic interactions between the mitogenic effects of vasopressin and TPA, in the presence or absence of insulin or EGF. Cultures produced as in Fig. 1 legend were washed twice in DMEM, and incubated in 2 ml of a 1:1 mixture of DMEM: Waymouth medium containing ^3H -TdR plus the mitogens as indicated. After 40 h at 37 °C, cumulative ^3H -TdR incorporation into acid-insoluble material was measured. Conditions and measurements of ^3H -TdR incorporation were as in Fig. 1. Fetal calf serum (10%) induced incorporation of 156×10^3 and 105×10^3 c.p.m. in the experiments in a and b, respectively. Concentrations of mitogens were 1 $\mu\text{g ml}^{-1}$, 100 ng ml^{-1} , 10 ng ml^{-1} and 10 ng ml^{-1} for insulin, TPA, EGF and vasopressin, respectively. a; ●, TPA plus insulin plus vasopressin; ▲, TPA plus insulin; ▲, TPA plus vasopressin; ■, TPA only. b; ▼, vasopressin + TPA + EGF; ■, vasopressin plus EGF; ●, vasopressin plus TPA; ▲, vasopressin only.

of various agents, including EGF, FDGF, insulin, unfractionated serum (Table 1), retinoic acid and colchicine (Fig. 1).

No additive or synergistic stimulation of DNA synthesis

The striking similarity in the mitogenic properties of TPA and vasopressin in Swiss 3T3 cells raises the possibility that they act through a common mechanism. If they stimulate DNA synthesis by an identical pathway, these chemically diverse factors should not interact either additively or synergistically in stimulating DNA synthesis when tested at saturating concentrations. We therefore tested their combined effect.

TPA caused a potent dose-dependent stimulation of ^3H -TdR incorporation into acid-precipitable material when tested in the presence of insulin (Fig. 2a). In contrast, addition of different concentrations of TPA in the presence of a fixed and saturating concentration of vasopressin (10 ng ml^{-1}), in either the absence

Table 1 Identical mitogenic properties of saturating concentrations of vasopressin and TPA

^3H -TdR (c.p.m. $\times 10^{-3}$)						
EGF (ng ml^{-1})	Insulin ($\mu\text{g ml}^{-1}$)	FDGF ($\mu\text{g ml}^{-1}$)	Serum % vol.:vol.	No other addition	Vaso- pressin	TPA
—	—	—	—	1	1	1
10	—	—	—	1	46	52
—	1	—	—	5	164	172
—	—	11	—	6	180	188
10	—	3	—	18	153	178
—	1	3	—	18	208	214
—	—	—	2.4	9	152	138
10	—	—	1.5	45	195	196
—	1	—	1.5	104	196	214

Cultures produced as for Fig. 1 were washed twice in DMEM and incubated for 40 h at 37 °C in 2 ml of a 1:1 mixture of DMEM: Waymouth medium containing ^3H -TdR (0.5 $\mu\text{Ci ml}^{-1}$; 1 μM) plus mitogens as indicated. Cumulative incorporation of ^3H -TdR was measured as in Fig. 1. Fetal calf serum (10%) induced incorporation of 208×10^3 c.p.m. Concentrations of vasopressin and TPA were 10 ng ml^{-1} and 100 ng ml^{-1} , respectively, which are saturating under these conditions (Fig. 2). FDGF was prepared as before⁴⁶.

or presence of insulin, did not alter stimulation of DNA synthesis (Fig. 2a). This lack of synergistic interaction between TPA and vasopressin was confirmed when both were added at submaximal concentrations together with insulin (results not shown).

Because the stimulation of DNA synthesis by saturating levels of TPA plus insulin or vasopressin plus insulin was frequently comparable to that promoted by fresh medium containing 10% serum (that is, maximal response), the possibility remained that a synergistic effect between TPA and vasopressin could be revealed in the presence of a third mitogen if DNA synthesis was stimulated submaximally. Figure 2b shows that in the presence of EGF the stimulation of DNA synthesis induced by saturating levels of TPA (a submaximal response) was not enhanced by increasing concentrations of vasopressin. Thus, vasopressin and TPA do not interact synergistically or additively in the presence or absence of a third mitogen.

The conclusions drawn from Fig. 2 are further substantiated by flow cytofluorometric analysis (Table 2). Vasopressin or TPA alone caused little increase in the percentage of the cell population moving from G_1 to $S+G_2+M$. Vasopressin and TPA together did not interact to increase cell exit from G_1 . However, the addition of vasopressin or TPA together with insulin caused a striking increase in the proportion of cells in $S+G_2+M$ which was not further increased when the three mitogens were added together in the culture. These results agree well with those in Table 1 and Fig. 2.

Table 2 Equivalence of mitogenic effects of TPA and vasopressin demonstrated by cytofluorometry

Vasopressin	TPA	Insulin	Serum	Percentage of cells in $S+G_2+M$
-	-	-	-	9
+	-	-	-	13
-	+	-	-	12
+	+	-	-	14
-	-	+	-	12
+	-	+	-	78
-	+	+	-	76
+	+	+	-	71
-	-	-	+	87

Cell cultures (90-mm) produced as for Fig. 1 were washed twice in DMEM and incubated for 40 h at 37 °C in 10 ml of a 1:1 mixture of DMEM:Waymouth medium containing 2 μ M colchicine (to arrest cells at mitosis) plus mitogens as indicated. Cells were then detached by minimal treatment with 0.025% trypsin and EDTA (0.16 mg ml⁻¹), suspended in DMEM containing 10% bovine serum, centrifuged at 1,000 r.p.m. for 2 min, washed twice with phosphate-buffered saline, suspended in 1 ml of saline and squirted rapidly through a 25-gauge needle into 3 ml of ethanol:saline (70:30). This suspension could be stored at 4 °C for 1–2 weeks. Immediately before a cytofluorometric assay, the ethanolic suspension was centrifuged at 1,000 r.p.m. for 2 min and the pellet was resuspended in 0.5 ml of a mixture containing mithramycin (50 μ g ml⁻¹) in ethanol:H₂O (25:75) plus 15 mM MgCl₂. After 30 min DNA histograms were obtained with a fluorescence activated cell sorter. Two 90-mm cultures were used for each condition. Concentrations of mitogens were 10 ng ml⁻¹, 100 ng ml⁻¹, 1 μ g ml⁻¹ and 10% (volume:volume) for vasopressin, TPA, insulin and fetal calf serum, respectively.

In all the above experiments, cumulative DNA synthesis was measured after a 40-h exposure of cultures to mitogens. It is possible that vasopressin or TPA, in the presence of insulin and other agents, induce identical amounts of DNA synthesis but with different kinetics. The experiment shown in Fig. 3 rules out this possibility. Both the lag period preceding the onset of DNA synthesis (14 h) and the rate of entry into S phase were the same regardless of whether the cultures were exposed to TPA plus insulin, vasopressin plus insulin or TPA plus vasopressin plus insulin. Thus, with respect to the kinetics of stimulation of DNA synthesis as well as cumulative DNA synthesis, the effects of vasopressin and TPA are neither additive nor synergistic. Note that serum-stimulated cells enter S phase more rapidly than the parallel cultures treated with the tumour promoter and the hormones, indicating that potential additive or synergistic effects could be detected under our experimental conditions.

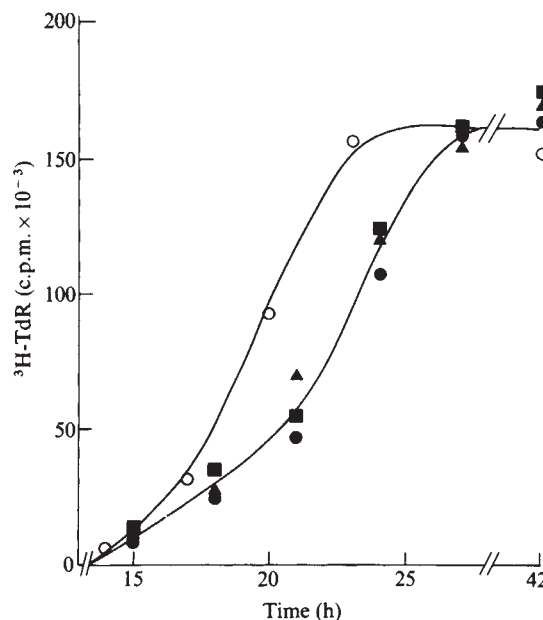


Fig. 3 Time course of ³H-TdR incorporation into Swiss 3T3 cells stimulated by different combinations of TPA, vasopressin and insulin. Cultures produced as in Fig. 1 legend were washed twice in DMEM. Then 2 ml of a 1:1 mixture of DMEM:Waymouth medium containing ³H-TdR plus mitogens as indicated was added (time 0), and the cultures were incubated at 37 °C. At the times indicated on the abscissa the incubation of duplicate cultures for each condition was stopped. The cumulative incorporation of ³H-TdR into the acid-insoluble pools of these cultures was then determined. Conditions and measurements of incorporation were as for Fig. 1. Insulin, TPA or vasopressin alone induced incorporation of less than 15 × 10³ c.p.m. of ³H-TdR. Concentrations of mitogens were 10% (volume:volume), 10 ng ml⁻¹, 1 μ g ml⁻¹ and 100 ng ml⁻¹ for fetal calf serum, vasopressin, insulin and TPA, respectively. ●, Vasopressin plus insulin; ■, TPA plus insulin; ▲, vasopressin plus TPA plus insulin; ○, serum.

The above evidence shows that vasopressin and TPA stimulate DNA synthesis by a common mechanism in Swiss 3T3 cells. However, TPA is a potent mitogen for several cell lines in which vasopressin is not active. For example, TPA stimulates DNA synthesis in quiescent 3T6 and hamster Nil-8 cells and synergistically stimulates DNA synthesis in the presence of other growth factors in BALB 3T3 cells^{16,21}. Vasopressin when added to these cells fails to enhance DNA synthesis or elicit any early biological response either in the presence or absence of other growth factors (unpublished data). It seems likely that these cell lines possess receptors for TPA (see below) but not for vasopressin, which in turn suggests that TPA and vasopressin interact with distinct receptors in Swiss 3T3 cells.

Stimulation of ornithine decarboxylase activity and 2-deoxyglucose uptake

Do the actions of TPA and vasopressin converge before the initiation of DNA synthesis? When quiescent cultures of 3T3 cells are stimulated by serum or by defined growth factors, a complex array of biochemical events precedes the onset of DNA synthesis²³. Two of these events—the induction of ornithine decarboxylase activity and of 2-deoxyglucose uptake—both of which peak 6 h after stimulation, are synergistically increased by combinations of growth factors. These events provide useful markers to determine whether the actions of TPA and vasopressin converge before the initiation of DNA synthesis, which occurs 14 h after mitogenic stimulation.

The activity of ornithine decarboxylase, a key enzyme in the biosynthesis of polyamines, rises markedly in different cell types stimulated by TPA^{24–26} and the induction of this enzyme has been proposed as essential for tumour promotion²⁷.

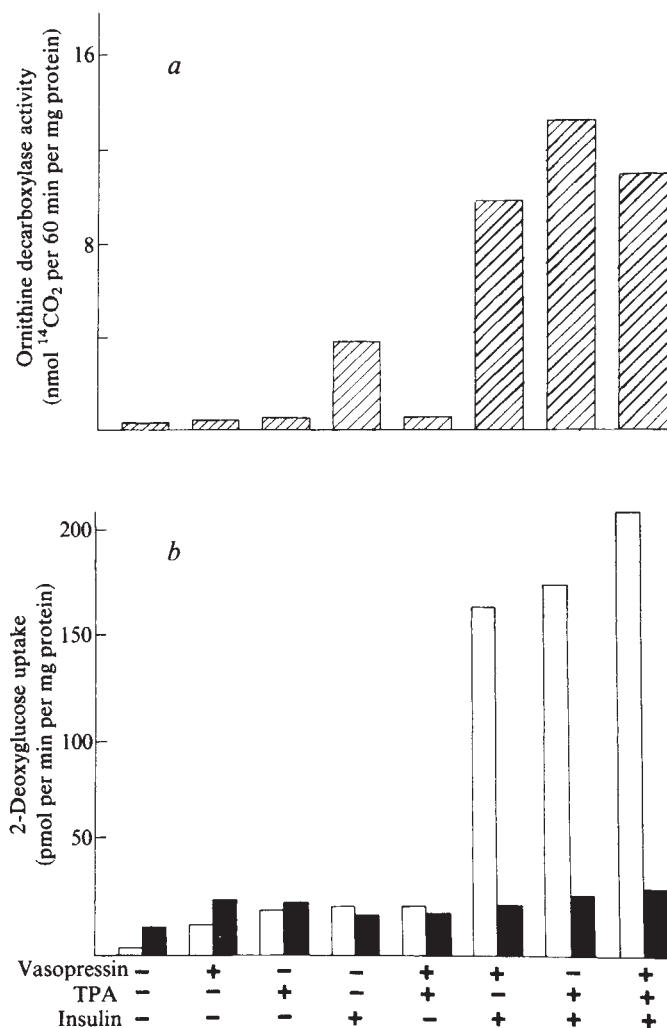


Fig. 4 Effect of TPA and vasopressin on ornithine decarboxylase activity (a) and ³H-2-deoxyglucose uptake (b) in the absence and presence of insulin. a, Quiescent 90-mm cultures of Swiss 3T3 cells produced as for Fig. 1 were washed twice in DMEM and incubated for 6 h in 10 ml of a 1:1 mixture of DMEM:Waymouth medium plus TPA or the hormones as indicated. Cultures were then washed three times in ice-cold saline and cells were scraped off the dish with a rubber policeman. For each experimental condition, cells from three separate cultures (about 10⁷ cells in total) were pooled, spun down, resuspended in 1 ml of saline, then spun down once more and stored at -40° until assayed. The assay used was similar to that of Hogan⁴⁴. The cell pellet was frozen and thawed three times in 200 μ l of the assay mixture. The final concentration of the various components in the enzyme assay was 0.1 mM EDTA, 0.05 mM pyridoxal phosphate, 5 mM dithiothreitol, ¹⁴C-ornithine (0.2 μ Ci; 0.2 mH, Amersham) and 50 mM of HEPES, pH 7.1. The release of radioactive CO₂ in the assay was linear for at least 60 min of incubation at 37 °C and was also proportional to the amount of cell extract used. The amount released did not vary by more than 10% in duplicate samples and the values presented are the average of duplicate determinations. To ensure that the release of ¹⁴CO₂ from ¹⁴C-ornithine was a valid measure of enzymatic activity, the formation of ³H-putrescine (Amersham) from [5-³H]ornithine was determined. Labelled putrescine was isolated from the reaction mixture and analysed by the method of Inoue and Mizutani⁴⁵. The amount of ³H-putrescine formed was proportional to the amount of ¹⁴CO₂ released. Concentrations of mitogens were 10 ng ml⁻¹, 100 ng ml⁻¹ and 1 μ g ml⁻¹ for vasopressin, TPA and insulin, respectively. b, Confluent quiescent 30-mm cultures produced as for Fig. 1 were washed twice in DMEM and incubated for 5.5 h in 2 ml of a 1:1 mixture of DMEM:Waymouth medium with the mitogens as indicated in the presence (black bars) or absence (plain bars) of 10 μ g ml⁻¹ cycloheximide. The cultures were then washed three times in DMEM minus glucose and incubated in DMEM minus glucose plus the mitogens as indicated with or without cycloheximide. After 30 min the cultures received ³H-2-deoxyglucose (2.5 μ Ci ml⁻¹; 0.2 mM Amersham). After 15 min at 37 °C, cultures were washed rapidly four times with ice-cold saline. Acid-soluble radioactivity was measured by extracting for 20 min with 5% TCA at 4 °C. All values are the mean of duplicate determinations. Concentrations of mitogens were as for a.

Vasopressin, like TPA, increases the activity of ornithine decarboxylase²⁶. Figure 4a shows that whereas addition of TPA or vasopressin to quiescent 3T3 cultures in the absence of other mitogens caused little increase in the activity of this enzyme, in the presence of insulin saturating concentrations of TPA or vasopressin synergistically induced identical levels of ornithine decarboxylase. When added together, the promoter and the hormone did not act synergistically in increasing enzyme activity in either the absence or presence of insulin. Thus the actions of vasopressin and TPA converge before the initiation of DNA synthesis.

The uptake of 2-deoxyglucose increases in a biphasic fashion^{23,28-30} in quiescent fibroblasts stimulated to divide. In various types of cells TPA induces an increase in 2-deoxyglucose uptake^{31,32}. In 3T3 cells TPA and insulin added together synergistically induced the cycloheximide-sensitive (second) phase of deoxyglucose uptake (Fig. 4b). Vasopressin could replace TPA in inducing the activity of this uptake system, acting synergistically with insulin. However, TPA and vasopressin together failed to act either synergistically or additively in the absence or presence of insulin. Thus, as with ornithine decarboxylase induction, TPA and vasopressin induce the second phase of 2-deoxyglucose uptake by a common mechanism.

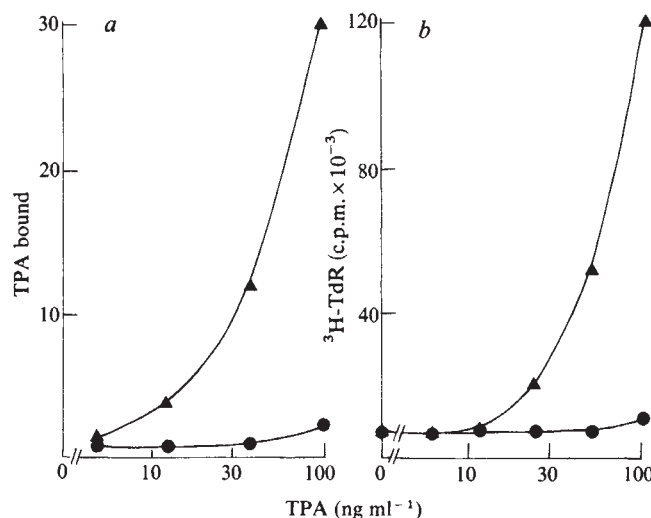


Fig. 5 Physical persistence of TPA (a) and the persistence of its mitogenic actions (b) are eliminated in parallel by BSA. a, 30 mm cultures produced as for Fig. 1 were washed twice in DMEM and incubated for 1 h at 37 °C in 2 ml of a 1:1 mixture of DMEM:Waymouth medium containing insulin (1 μ g ml⁻¹) plus [20-³H(N)]TPA (0.15 μ Ci ml⁻¹, NEN) at various specific activities. Cultures were then washed twice with DMEM at 37 °C and the amount of TPA bound (ng per culture) was determined from the remaining radioactivity extracted as described below ($\blacktriangle$). Parallel cultures were exposed three successive times (45 min each) to 2 ml of recrystallized defatted BSA (10 mg ml⁻¹, Sigma) in a 1:1 mixture of DMEM:Waymouth medium supplemented with insulin (1 μ g ml⁻¹), followed by three 10-min washes with the same medium without BSA ($\bullet$). Radioactivity was extracted from the dish after two rapid washes with ice-cold 0.15 M saline followed by a 30-min incubation at 37 °C with 0.1 M NaOH and 1% SDS. Insulin was present in these incubations to maintain cell attachment. The number of cells per culture was the same after either treatment. The ³H-TPA was dissolved in ethanol at a final concentration of less than 0.2% which had no effect on the cell cultures. b, Cultures were washed twice in DMEM and incubated for 1 h at 37 °C in 2 ml of a 1:1 mixture of DMEM:Waymouth medium containing insulin (1 μ g ml⁻¹) plus various concentrations of TPA. Cultures were washed twice in DMEM and 2 ml of a 1:1 mixture of DMEM:Waymouth medium containing insulin (1 μ g ml⁻¹) and ³H-TdR (0.5 μ Ci ml⁻¹; 1 μ M) was added either immediately ($\blacktriangle$) or after the cultures had been washed with BSA solutions as above ($\bullet$). After 40 h at 37 °C cumulative incorporation of ³H-TdR into acid-insoluble pools was assayed as for Fig. 1. Addition of TPA (100 ng ml⁻¹) and insulin (1 μ g ml⁻¹) to cultures pretreated with BSA or with cultures not pretreated with BSA, induced incorporation of 118 $\times 10^3$ and 142 $\times 10^3$ c.p.m. of ³H-TdR, respectively, after a 40-h incubation. This treatment with BSA does not cause cells to become less responsive to the mitogenic actions of TPA plus insulin. Fetal calf serum (10%) induced incorporation of 185 $\times 10^3$ c.p.m. in this experiment.

Table 3 TPA acts synergistically with each of a wide range of mitogens except vasopressin

Mitogens acting synergistically with TPA	Reference
Serum	4, 13
Depleted serum	14
EGF	10, 15, 16
Insulin	15, 16
FDGF	16
Fibroblast growth factor	15
Platelet-derived growth factor	15
Platelet-poor plasma	15
Multiplication-stimulating activity	15
Antitubulin agents	This report
Retinoids	21
Mitogens unable to act synergistically with TPA	
Vasopressin	This report

Transient exposure to TPA

The mitogenic effects of TPA persist after the removal of the TPA-containing medium (refs 15 and 16 and our unpublished data), but vasopressin must be in contact with cells continuously to stimulate DNA synthesis synergistically with insulin¹⁷. It has been suggested that mitogens such as TPA, which exert biological effects that persist after the medium containing the mitogen has been removed, act by inducing a cell to become 'competent'³³. Mitogens such as vasopressin, which must be present throughout G₁ to cause entry into S phase¹⁷, are postulated in this model to act in a fundamentally different fashion: they are said to allow a 'competent' cell 'to progress' through G₁ and into S phase³³. According to this model TPA and vasopressin act by separate paths. This contradicts the evidence reported here. However, although the biological effects of TPA may be mediated by specific receptors (see below), it is also known that TPA associates tightly but nonspecifically with cell membranes³⁴, a phenomenon which may account for its persistent biological effects and has impeded the detection of specific binding of the molecule. The following experiments show that the mitogenic effects of TPA persist after the removal of the TPA-containing medium, merely because it binds tenaciously to cell cultures. Figure 5a demonstrates that the phorbol ester remains bound to cell cultures even after multiple washes with Dulbecco's modified Eagle's medium. About 30% of the ³H-TPA added in a 1-h incubation remained attached to the culture after washing and this was not reduced by adding a vast excess of unlabelled TPA (that is, binding was nonspecific). TPA binds with high affinity to bovine serum albumin (BSA)³⁴, and the level of TPA remaining bound to cell cultures could be reduced considerably by repeated incubation with solutions of BSA (Fig. 5a). If the ability of TPA to exert its mitogenic effects after removal of the TPA-containing medium is due merely to its remaining bound to the cell culture, incubations with BSA solutions should prevent the persistence of the mitogenic effects of TPA. Figure 5b shows that this is indeed the case. The removal of TPA from cell cultures by BSA (Fig. 5a) parallels the elimination of the persistent mitogenic effects of TPA (Fig. 5b). Thus, as with vasopressin, a transient exposure to TPA is not sufficient to render the cells responsive to insulin in stimulating

DNA synthesis. This is consistent with our data showing that vasopressin and TPA act as mitogens by identical mechanisms.

Conclusions and implications

Our study demonstrates that (1) TPA and vasopressin can substitute for each other in stimulating DNA synthesis, ornithine decarboxylase induction and 2-deoxyglucose uptake under various experimental conditions; (2) TPA and vasopressin added at saturating concentrations do not have additive or synergistic effects in eliciting these responses in either the absence or the presence of other factors such as EGF or insulin. The lack of synergistic interaction between TPA and vasopressin sharply contrasts with the synergistic interaction of TPA with practically all known growth-promoting polypeptides as summarized in Table 3. These findings indicate that vasopressin and TPA control cellular responses in mouse 3T3 cells by a common mechanism.

The effects on the induction of ornithine decarboxylase activity and 2-deoxyglucose uptake suggest that the common step occurs rapidly after TPA and vasopressin interact with the cell, possibly at the level of the cell membrane. There is considerable evidence that the membrane is an important target for the biological actions of TPA^{31,35,36}. TPA can rapidly trigger ion fluxes in responsive cells (ref. 35 and our unpublished data) and similar findings have been made with vasopressin³⁷. Another rapid and striking effect of TPA on the cell membrane is an alteration in the receptors for ¹²⁵I-EGF, which results in a profound inhibition of growth factor binding^{8,10-12}. Recently we have found that vasopressin induces a potent, reversible and temperature-dependent change in the affinity of cellular receptors for EGF, again suggesting that the biochemical effects of TPA and vasopressin converge at the level of the plasma membrane⁴⁷.

Vasopressin and TPA could bind to specific receptors in 3T3 cells because their effects are elicited at low concentrations, are saturable and exhibit remarkable stereospecificity^{16,17}. Indeed, specific binding of phorbol dibutyrate (a tumour-promoting phorbol ester) to membrane preparations of chick fibroblast has recently been reported³⁸ and there is physiological evidence for at least two classes of vasopressin receptors³⁹. In theory, the lack of additive or synergistic interactions between saturating concentrations of TPA and vasopressin may result from (1) both agents binding to an identical cellular receptor; (2) both compounds binding to separate sites but interacting with a common effector system, or (3) TPA directly modulating the activity of a coupling protein⁴⁰ or an effector system that can interact with vasopressin receptors in those cells which possess such receptors. The lack of apparent chemical analogy between these agents and the fact that in several cell types TPA is mitogenic whereas vasopressin is biologically inactive (presumably because these cells do not express vasopressin receptors) renders the first possibility unlikely. More data are required to distinguish between the second and third alternatives, although it is interesting that TPA uncouples β -adrenergic receptors from adenyl cyclase in mouse epidermis^{41,42}. Regardless of the specific details, the recognition that the phorbol ester tumour promoters act at least in part through a specific hormonal pathway is a prerequisite to understanding the mechanism of action of these important molecules.

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LETTERS

Is PKS1921-29 a quasar with correlated radio and optical variations?

Gerard Gilmore

Royal Observatory, Blackford Hill, Edinburgh EH9 3HJ, UK

Although many quasars and related objects are variable at both optical and radio frequencies, the relationship, if any, between these variations is unclear. In particular, only three objects, OJ287 (ref. 1), 0235+164 (ref. 2) and 0420-01 (ref. 3), have shown well correlated radio-optical variability. The first two are BL Lac objects and showed little or no time delay between the radio and optical variations. The last object is a quasar, and underwent a radio flare some 2 years after a significant optical outburst. Optical observations of another quasar, PKS1921-29, which may also show correlated radio and optical variability are reported here.

Quasar PKS1921-29 has been monitored since 1975.6 at Mount John University Observatory of the University of Canterbury, New Zealand, as part of a larger quasar patrol programme^{4,5}. All 27 observations available were taken with an unfiltered S20R photocathode image intensifier camera (effective $\lambda \approx 550$ nm). The plates were measured on a Becker iris photometer at the Royal Observatory, Edinburgh, and data reduced as described elsewhere⁴. The results are shown in Fig. 1. The object is also visible on two ESO Blue Sky Survey plates (fields 459, 460) and five UK Schmidt Telescope plates (IIIaJ + GG395). Magnitudes for the object and the comparison stars used in the iris photometry were visually estimated on these plates, and are also shown in Fig. 1. The fortuitous presence of the globular cluster NGC6809 near the centre of ESO/SRC Survey field 460 allowed the magnitude scale to be calibrated by comparison with the photometry of Harris⁶. Because of the different bandpasses, however, the possibility of both zero point and scale differences between the two data sets remains, and care should be taken in applying the magnitude scale of Fig. 1 to the Canterbury results.

The significant features of these results are the large flare (≥ 1.5 mag) beginning near 1977.4, lesser amplitude activity in 1978, and the very bright state recorded during the single observation in 1979. By comparison, the object appears both fainter and less active during 1974-77, although few observations are available, and this may simply represent a sampling effect. Additionally, photoelectric observations on 9 May 1980 (UT) (not shown in Fig. 1) showed the object to have returned to near minimum level at $m_B = 16.8$ (P. Brand and C. Impey, personal communication).

Dent and Balonek⁷ have recently reported radio flux observations from 1972 to 1980 at four frequencies between 7.9 and 89.6 GHz. These show a steady decline in radio flux from 1972

to 1976, a more rapid increase to 1979.4, and then a very rapid increase continuing at least to 1980.0. Superimposed on these trends are small ($\pm 25\%$) flares near 1975.3 (predominantly seen at 15.5 GHz) and 1977.5 (seen only at 31.4 and 89.6 GHz). Landau *et al.*⁸ report 90 GHz data and note a factor of 2 decline in flux in only 2 days in December 1978 (see also ref. 9).

The coincidence of the increasing high frequency variability, and the optical variability may be evidence for a common origin of all activity. The significance of this cannot, however, be evaluated until the frequency of optical flaring, such as that in 1977, is better determined.

The optical burst beginning near 1977.4 is also significant. This may be correlated with the maximum of the high frequency activity (31.4 GHz and 89.6 GHz) reported by Dent and Balonek during 1977. In this case, however, the optical flare began near the maximum of the radio activity, and lasted for a significantly shorter time than the radio flare. Similar behaviour in the BL Lac type object AO0235+164 has recently been reported by Balonek and Dent¹⁰.

Note that two possible identifications for this radio source have been published. Radovich and Kraus¹¹ suggested a 16.5 mag red star, while Peterson *et al.*¹² give a different identification. The Radovich and Kraus identification has shown no statistically significant variability on any of the plates measured here. The light curve in Fig. 1 refers to the Peterson *et al.* identification, and supports that object as the correct optical counterpart to OV236. Final confirmation is provided by optical spectroscopy (ref. 13 and J. G. Bolton, personal communication) which provides a redshift $z = 0.352$ from two lines.

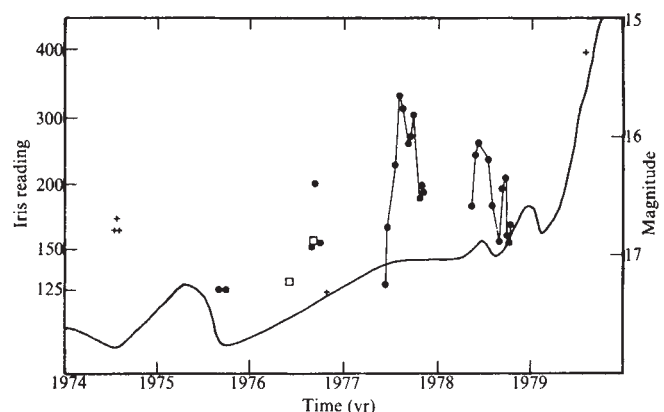


Fig. 1 Optical observations of PKS1921-29 at Mt John University Observatory (●, iris readings scale), on ESO B Survey plates (□) and UK Schmidt Telescope plates (+, magnitude scale). The magnitude scale is not accurately tied to the iris readings scale (see text). Photometric uncertainty is typically ± 0.1 mag. The solid line is a smoothed representation of the 15.5 GHz radio data reported by Dent and Balonek⁷.

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Star formation in a galactic wind

A. C. Fabian, P. E. J. Nulsen & G. C. Stewart

Institute of Astronomy, Madingley Road, Cambridge CB3 0HA, UK

Malin and Carter¹ have discovered giant shells of faint optical emission around several normal elliptical galaxies. We suggest here that these shells are composed of stars formed in shocked galactic wind material. Shocks might arise from relatively gradual increases in wind velocity, or from the collision of the wind with a tenuous intergalactic medium, and need not imply that cataclysmic events have occurred within the galaxy.

The evolution of the stars in an elliptical galaxy should produce a few $M_{\odot} \text{ yr}^{-1}$ of gas², mostly from giant stars and planetary nebulae. The lack of observable gas in most ellipticals has led to the suggestion^{3,4} that the gas produced there is driven out of the galaxy in a wind powered by supernovae. Typical wind velocities are estimated to be several hundred kilometres per second.

The wind expands freely beyond the edge of the galaxy, so that its temperature falls rapidly and the flow becomes highly supersonic. Thus when it passes through a shock of velocity $v_s = 10^7 v_7 \text{ cm s}^{-1}$ the gas is heated to a temperature

$$T_s = 1.3 \times 10^5 v_7^2 \text{ K} \quad (1)$$

The shocked gas is compressed to a density n_e electrons cm^{-3} and cools to $\leq 10^4 \text{ K}$ on a time scale

$$t_c = 1.4 \times 10^3 T^{1.6} n_e^{-1} \text{ s} \quad (\text{ref. } 5)$$

that is

$$t_c = 1.5 \times 10^8 R_{50}^2 \dot{M}^{-1} v_7^{4.2} \text{ yr} \quad (2)$$

behind a stationary shock at $R_s = 50 R_{50} \text{ kpc}$ in a (spherical) wind of $\dot{M} M_{\odot} \text{ yr}^{-1}$. The pressure of the shocked gas is maintained at

$$n_e T = 8.4 R_{50}^2 \dot{M} v_7 \text{ cm}^{-3} \text{ K} \quad (3)$$

by the ram pressure of the wind. The shock is easily confined by a very tenuous intergalactic (or circumgalactic) medium⁶ (for example, at a temperature $\sim 10^7 \text{ K}$ and density $\sim 8 \times 10^{-7} \text{ cm}^{-3}$, which is much less than the closure density of the Universe).

The total power in the wind is only $\sim 3 \times 10^{39} \dot{M} v_7^2 \text{ erg s}^{-1}$ and will be converted into optical (mostly line) radiation with an efficiency which decreases with increasing v_7 . Thus the cooling gas is unlikely to be visible.

The emission seen by Malin and Carter¹ is easily provided by $\sim 10^8 M_{\odot}$ of stars, each of $\sim 1 M_{\odot}$. Star formation can occur in the cooled gas if it is unstable to self-gravitation, that is Jeans

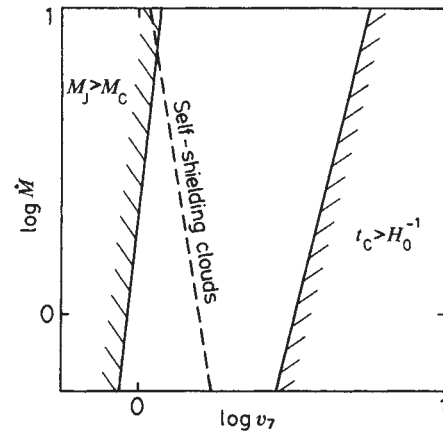


Fig. 1 $\dot{M}$ and v_7 are the mass loss rate and velocity of a galactic wind in $M_{\odot} \text{ yr}^{-1}$ and 10^7 cm s^{-1} respectively. Gas behind a shock in a wind at a radial distance of 50 kpc can cool within the age of the Universe provided that $\dot{M}$ and v_7 lie to the left of the rightmost line. The Rayleigh–Taylor instability causes cooled gas to form clouds of mass M_c , which exceed the Jeans mass M_J at 100 K and can thus collapse to form stars, if $\dot{M}$ and v_7 are to the right of the leftmost line. External shielding of quasar ionizing UV radiation is required if stars are to form below the dashed line.

unstable. To estimate whether this is possible we identify the likely length and mass scales that occur. The dense cooled gas is supported over lighter and more recently shocked gas against the pull of the galaxy. This situation is subject to the Rayleigh–Taylor instability which causes the shell to break into clouds of size r_c in a time

$$t_{\text{RT}} \approx t_{\text{ff}} (2/\pi)^{3/2} (r_c/R_s)^{1/2} \quad (4)$$

where the free-fall time into a galaxy of mass $5 \times 10^{11} M_{\odot}$,

$$t_{\text{ff}} = 2.6 \times 10^8 R_{50}^{3/2} \dot{M}^{-1/2} \text{ yr} \quad (5)$$

This fragmentation cannot proceed before the shocked gas has begun to cool, and leads to the size of a typical cloud forming at t_c being similar to the thickness of the shell of shocked gas (see ref. 7);

$$\begin{aligned} \Delta R &= \frac{1}{4} v_s t_c \\ &= 4 R_{50}^2 \dot{M}^{-1} v_7^{5.2} \text{ kpc} \end{aligned} \quad (6)$$

The corresponding cloud mass is then

$$M_c = 1.6 \times 10^5 R_{50}^4 v_7^{14.6} \dot{M}^{-2} M_{\odot} \quad (7)$$

The Jeans mass in neutral gas at a pressure given by equation (3) and a temperature $10^4 T_4 \text{ K}$ is

$$M_J \approx 5.0 \times 10^8 R_{50}^2 \dot{M}^{-1/2} v_7^{-1/2} T_4^2 M_{\odot} \quad (8)$$

which is less than M_c provided that the gas can cool still further to $T_4 \sim 10^{-2}$ and that

$$\dot{M} \leq 2.2 R_{50}^2 v_7^{10} M_{\odot} \text{ yr}^{-1} \quad (9)$$

The steep dependence on shock velocity means that gravitational collapse, and we presume star formation, can occur for almost any $\dot{M}$, provided that $v_7 \geq 1$ (see Fig. 1).

An important step in the above process is the cooling of clouds from 10^4 K to $\sim 10^2 \text{ K}$, so that the Jeans instability can occur. In the absence of any heat source in the clouds, this further drop in temperature can occur by radiative cooling due to the presence of heavy elements in the wind material. The only obvious heat source is ionizing UV radiation from quasars^{8,9}, which is poorly determined and may be shielded by gas at larger distances than are considered here. If no such external shield is present, the clouds at 10^4 K must shield themselves against the ionizing UV

flux, assumed to be $10^4 \Phi_4$ photons $\text{cm}^{-2} \text{s}^{-1}$, where $\Phi_4 \sim 1$ (refs 8, 9). This requires a cloud radius

$$r_{UV} \geq 39 \Phi_4 R_{50}^4 \dot{M}^{-2} v_7^{-2} \text{ kpc} \quad (10)$$

and we obtain $r_{UV} < r_c$ if

$$\dot{M} \geq 18 R_{50}^2 v_7^{-6.5} \Phi_4 M_\odot \text{ yr}^{-1} \quad (11)$$

(Fig. 1).

The details of the process of star formation are not well understood. However, there is a substantial range in $\dot{M}$ and v_7 (Fig. 1) for which Jeans unstable clouds can arise. We propose therefore that shells of $\geq 10^8 M_\odot$ of stars can sometimes form from galactic wind material at large distances from elliptical galaxies. The approximate spherical symmetry of the wind will be reflected in the distribution of the stars. To form a thin shell, the star formation must occur on a time scale which is short relative to the free-fall time. Patches of stars may form at several different radii following thermal instabilities in a thick region of shocked gas ($\Delta R \sim R$) or from velocity changes in the wind. These changes could be due to a cooling instability in the core of the galaxy⁴, or to more dramatic events such as the accretion of a gas-rich companion or an outburst of the nucleus. Continuous star formation at a fixed radius gives the appearance of a thin shell, as the acceleration of the stars from rest under the influence of the galaxy gives a density profile $\rho_* \propto$

$(R_s/R - 1)^{-1/2}$. Localized bursts of star formation in more peculiar galaxies might lead to small-scale wind shocks. The loop structures seen in Fornax A (NGC1316) could, in part, be due to this mechanism.

Star shells produced at large distances from a parent galaxy may remain roughly spherical for a reasonably long time scale $\sim t_{ff}$. This will be the approximate age of the component stars. We therefore expect the shells to appear bluer¹¹ than the parent galaxy (up to ~ 0.5 mag in $U-B$ and $B-V$) if the initial mass function is similar to that in the solar neighbourhood. Thereafter these high-velocity stars may contribute to the outer galactic envelope. Shocks in denser galactic winds at earlier epochs could have created substantial isothermal stellar envelopes to galaxies.

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Volcanic material from Mount St Helens in the stratosphere over Europe

M. Ackerman, C. Lippens & M. Lechevallier*

Institut d'Aéronomie Spatiale de Belgique, 3, Avenue Circulaire, B-1180 Brussels, Belgium

*E.T.C.A., 16 bis, Avenue Prieur de la Côte d'Or, F-94114 Arcueil, France

The two most recent balloon flights devoted to studying the stratospheric aerosol vertical structure by comparison with winds, temperature and ozone vertical structures took place on 7 May and on 5 June 1980. At 15.23 h GMT on 18 May Mount St Helens volcano (46°N , 122°W) erupted with a tremendous explosion, projecting ash into the stratosphere. An explosion of this size occurs only about once a decade¹. This sudden introduction of material into the atmosphere offers the opportunity to study air motions both horizontally and vertically. The last such large-scale opportunity was offered by the Mount Fuego eruption which took place in 1974. In this latter case, the enhancement of stratospheric aerosols was observed by means of ground-based lidars and of balloon-borne particle counters. The time development of the aerosol event in 1974 and 1975 has been described elsewhere^{2–4}. Mount St Helens material can now also be traced by various satellite-borne instruments. This new stratospheric aerosol event appears to be spectacular. As shown by the photographs discussed here, it leads in its early stage of development to an increase by a factor of three of the Earth limb reflectivity at 15-km altitude after a period of several years of low stratospheric aerosol content.

The new method⁵ is based on the photographic observation from balloon altitude of the Earth limb in the azimuthal direction of the Sun at low solar elevation angles. Aerosols are known to scatter light mostly at small angles whereas, comparatively, Rayleigh scattering by air molecules occurs almost evenly at all angles. Our observational geometry favours aerosol detection.

At balloon altitude the camera lenses view the Earth limb below the horizontal line directly and the Sun above it through a neutral optical density ($D \approx 4$) screen which serves several purposes. It avoids penetration of straylight in the optics to obtain clean pictures of the Sun at various elevation angles providing an angular scale. It puts both the Sun and the Earth

limb in the sensitive dynamic range of the film calibrated in relative units by means of a step wedge photographed before flight. The solar images provide the absolute scale. The gondola is Sun oriented so that the cameras have the Sun in the middle of the horizontal field of view. Colour (Kodak EPR 475) and black

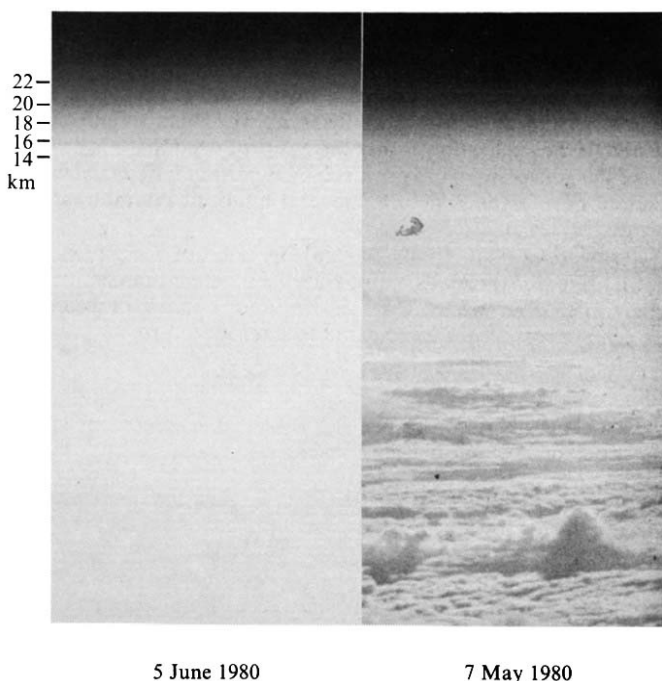


Fig. 1 Photographs of the Earth limb taken with identical exposure settings in the azimuth of the Sun at low solar elevation angles from balloon gondolas on 7 May and on 5 June 1980. The gondolas were at 37- and 35-km altitude respectively. An altitude scale of the grazing line of sight is shown in kilometres. Below the dark blue sky horizontally stratified aerosol layers are revealed by scattered sunlight. In the 5 June case, material from the Mount St Helens volcano has spread in the low stratosphere over a large horizontal extent. It enhances the brightness of the limb so that the cloud cover in the foreground can hardly be seen through the whitish veil. In contrast, the cloud cover is well visible on 7 May. Note that the grazing point at 16-km altitude is some 500 km away from the camera. An altitude difference of 1 km at that altitude corresponds to 7 arc min.

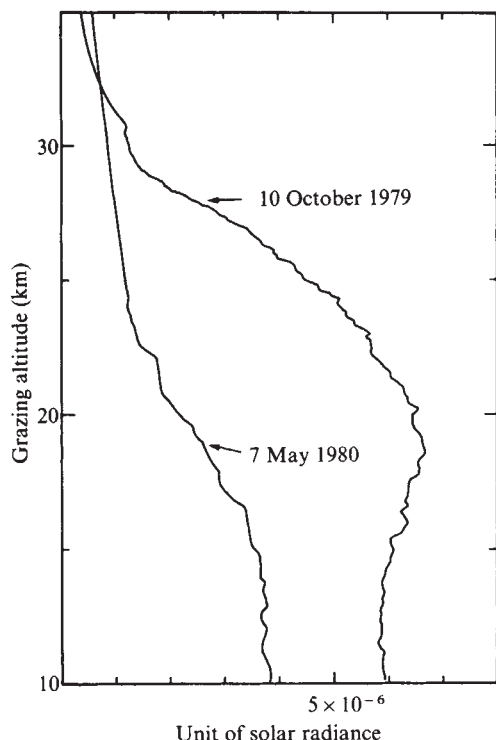


Fig. 2 Earth limb radiance in the azimuth of the Sun versus grazing altitude of the line of sight. The solar elevation angles were respectively 6.18° and 6.94° on 10 October 1979 and on 7 May 1980. The variation of the limb radiance versus altitude is relatively smooth in both cases. As most of the radiance observed is due to scattering by particles, their amount in the low stratosphere appears larger in October than in May where a low aerosol background had been reached. The situation at higher altitudes seems to be reversed. However, these aspects require confirmation from other flights and further interpretation of these raw data where ozone and air absorption should be taken into account. The uncertainties due to difficulties of the photographic photometry have also to be considered.

and white (Kodak Plus X Pan) 70-mm films are used. The results reported here were obtained in the red using Wratten filters nr. 25 set in front of the 80-mm focal length lenses of the Hasselblad EL500 cameras and without filter. The cameras are remotely operated from the ground. The telemetry return signal allows us to check their operation.

Three flights are considered here. The first two took place on the afternoons of 10 October 1979 and 7 May 1980 from Aire sur l'Adour in south-west France. To determine the geographical coordinates as accurately as possible, the tracking was performed by the CNES telemetry station and by radars located at the Centre d'Essais des Landes (Biscarosse). The altitude, longitude and latitude of the gondola at the time that the picture was taken were respectively 37 km, 1° E and 44° N. The third flight took place on 5 June 1980 from the other CNES range in the Alps. Radar tracking was performed in this case from Ile du Levant on the edge of the French Riviera. The coordinates were 35.2 km, 2° E and 44° N.

Inspection of limb pictures such as those shown in Fig. 1 reveals the presence of aerosol layers with a thickness ranging from 100 metres to several kilometres. The latter layers extend horizontally over the whole field of view whereas the thinner layers may have a smaller extension. The black and white photographs are analysed using a Jarrell-Ash densitometer. Grazing altitudes in the stratosphere are determined using the tables of Link and Neuzil⁶, taking refraction into account.

The Earth limb radiance is deduced from the densitometric measurements. The radiance vertical profiles observed on 10 October 1979 and on 7 May 1980 in white light are compared in Fig. 2. These profiles are similar showing mostly smooth vertical

structure. Figure 3 shows the vertical radiance profiles observed in red light on 7 May and on 5 June 1980. The abrupt radiance increase observed on 5 June between 15- and 16-km altitude is similar to the increase of particle amount observed shortly after the Fuego eruption²⁻⁴. The very sharp cutoff at the upper boundary of the layer is typical.

On the other hand, satellite-borne instruments have tracked at 16-km altitude material from the volcano crossing the east coast of the US on 23 May and moving towards Europe above the Atlantic Ocean on 27 May (M. P. McCormick, personal communication). The observation reported here is likely to have occurred shortly after the arrival over Europe.

Theory^{7,8} predicts that a stratospheric aerosol increase reduces the amount of solar energy reaching the troposphere and the ground. Two main processes are involved: absorption in

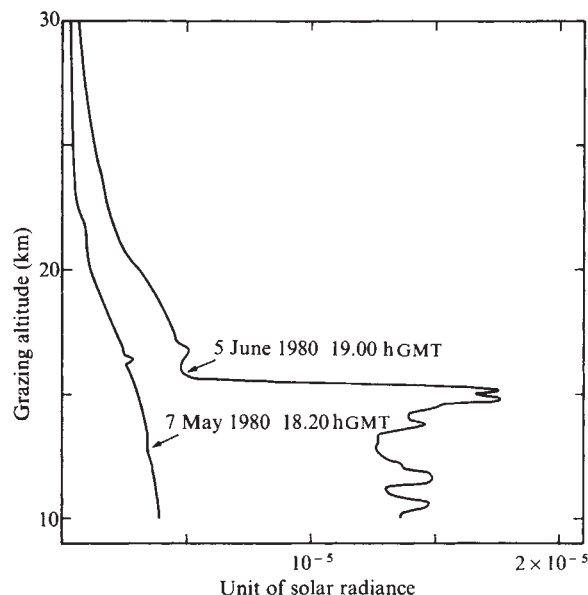


Fig. 3 Earth limb radiance in the azimuth of the Sun and in units of solar radiance versus altitude of the grazing line of sight. The solar elevation angles were respectively 6.94° and 6.26° on 7 May and on 5 June. The large and sharply cut radiance increase between 15.6 and 15.2 km is due to the stratospheric loading of volcanic material. The radiance increase at higher altitudes is thought to be partly due to the illumination increase of the upper levels by the lower levels. The thin layers (some 300-m thick) at some 16.5-km altitude seems to be a frequent feature of the mid-latitude stratosphere and is thought to be related with the height of the tropical tropopause.

the aerosol layer and reflection to space of solar energy flux leading to small average temperature decreases in the troposphere^{4,9}. The reflectivity data reported here will be augmented by further flights and together with other absorption data will hopefully improve the understanding of the problem and help to evaluate more precisely the possible impact of stratospheric aerosol events.

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Magnetic activity and variations in solar luminosity

E. A. Spiegel

Department of Astronomy, Columbia University, New York, New York 10027

N. O. Weiss*

Harvard-Smithsonian Center for Astrophysics, Cambridge, Massachusetts 02138

Attempts to detect changes in the solar luminosity suggest that the solar constant has been misnamed¹⁻⁴. Although the Nimbus satellite data⁴ show no significant fractional variations above 5×10^{-3} during the period 1975-78, results from recent balloon⁵ and rocket⁶ flights show changes of 4×10^{-3} . Intense magnetic fields in sunspots hamper convection locally⁷ but active regions are believed not to be directly responsible for long term variations in luminosity of the Sun⁸ or of RS CVn and BY Dra stars⁹. The cause of luminosity variations over spot cycles should be sought in more deep-seated global features. Here we indicate how strong magnetic fields at the base of the convective zone can alter the local convection. The resulting changes in thermal energy are large enough to produce variations of order 0.1% in the solar luminosity over the 11-yr sunspot cycle.

The magnetic field in the solar envelope is believed to be predominantly toroidal and generated by differential rotation^{11,12}. Flux expulsion and magnetic buoyancy^{11,12} will quickly sweep toroidal fields from the convective zone. Moreover, the observed large scales of coronal holes and complexes of solar activity point to a confinement of the toroidal field in the lower part of the convective zone¹³. We are led to a model in which the toroidal field is concentrated into a boundary layer at the base of the convective zone where there is relatively little motion. Flux expulsion and topological pumping¹¹ compress the fields into such a layer, and any flux tubes that stray into the main body of the convective zone will be brought up to the surface by magnetic buoyancy. Such a magnetic layer is deeply implicated in the solar cycle and is accordingly time dependent. We shall explore its influence on convective heat transfer.

Let T be the local temperature and $\bar{T}$ be its average over an equipotential surface, presumed spherical. Then $\bar{T}$ depends only on radius, r , and time. In the mixing-length treatment of stellar convection the heat flux is determined by the gradient of potential temperature,

$$G_p = \partial \bar{T} / \partial r - (\partial \bar{T} / \partial r)_{ad} \quad (1)$$

The adiabatic temperature gradient is given by

$$G_a = (\partial \bar{T} / \partial r)_{ad} = (\partial \bar{T} / \partial \bar{p})_{\bar{s}} \partial \bar{p} / \partial r = (\bar{p} C_p)^{-1} \partial \bar{p} / \partial r \quad (2)$$

where $\bar{s}$ is the horizontal average of the specific entropy. The radiative flux is fixed by the mean temperature gradient, $\partial \bar{T} / \partial r$.

From equation (2) and the hydrostatic relation,

$$\partial \bar{p} / \partial r = -g \bar{p} + \hat{r} \cdot [(\nabla \wedge \mathbf{B}) \wedge \mathbf{B} / (4\pi) - \nabla \cdot (\rho \mathbf{u} \mathbf{u})] \quad (3)$$

where $\mathbf{u}$ is velocity and $\hat{r}$ is a unit radial vector, it follows that G_a is directly affected by magnetic fields. Because the field $\mathbf{B}$ is solenoidal,

$$G_a = -g / C_p - (\bar{p} C_p)^{-1} \partial [(\bar{B}_h^2 - \bar{B}_v^2) / (8\pi) - \rho w^2] / \partial r \quad (4)$$

where B_h and B_v are the horizontal and vertical components of $\mathbf{B}$, and w is the vertical component of $\mathbf{u}$. In the nether regions of the convective zone $G_p \sim 10^{-6} \text{ K km}^{-1}$ (ref. 10). The magnetic correction produced by a toroidal field occupying a layer of thickness d is

$$\delta G_a \sim \bar{B}^2 / (8\pi \bar{p} C_p d) \quad (5)$$

with $\bar{p} \sim 0.1 \text{ g cm}^{-3}$ near the bottom of the solar convective zone. To estimate the variation in G_a during the solar cycle that results from the reversal of the toroidal field at sunspot minimum and the subsequent growth of magnetic flux with the approach of sunspot maximum, we need to focus on the magnetic layer that is formed between the radiative and convective zones by flux expulsion.

In standard mixing length theory, the bottom of the convective zone is well defined: motion is assumed to stop at the depth where $G_p = 0$. In reality, fluid slows down as it approaches this depth from above; then it overshoots and goes below it. A sublayer of mild convection forms in the lower part of which the toroidal field develops and whence it intrudes into the stably stratified radiative zone. In this magnetic intrusive region, $\partial B^2 / \partial r > 0$. Hence the adiabatic gradient is modified in a way that causes the bottom of the convective zone slowly to recede. This helps stabilize the layer in which magnetic field is building up.

The field grows more rapidly than the stable layers can admit it by diffusion. Flux expulsion drives the magnetic energy density up to values comparable with the kinetic energy density at the base of the convection zone. Hence, in the reclaimed part of the convective zone, fields will grow to values comparable with the equipartition value of 10^4 G . Hydromagnetic convection theory indicates several possible instabilities, both linear and nonlinear, involving magnetic buoyancy. These will be of the 'double diffusive' type, with stable temperature gradient and unstable magnetic gradient. According to the rough criteria available these instabilities occur when d , the thickness of the magnetic layer, approaches the pressure scale height, $H_p \sim 5 \times 10^4 \text{ km}$.

Instabilities driven by magnetic buoyancy produce ropes of magnetic flux, which rise up through the convective zone and emerge into the photosphere. As the subconvective fields become unstable, therefore, active regions appear more frequently at the surface and the sunspot cycle approaches a new maximum. The magnetic flux of a field of 10^4 G in a layer whose depth is of the order of H_p should, in this picture, be comparable with the flux that protrudes from the solar surface near sunspot maximum. This conclusion accords with observation¹⁴.

The onset of the instability affects the toroidal field strength and hence modifies the adiabatic gradient. Thus it is accompanied by an increase in the convective intensity in the lower convective zone. We suggest that these changes will give rise to variations in the solar luminosity. To gauge their amounts we may use our estimates of B^2 and d and find from equation (5) that $\delta G_a \sim 10^{-6} \text{ K km}^{-1}$, which is comparable with G_p . Other terms of like magnitude also occur in the heat equation, through which G_p has a central role in the theory. None of these terms is included in the usual mixing length theory of stellar convection and their precise relative magnitudes and phases are not known. Moreover, fields of 10^4 G must exert dynamical effects on the motion. From the rate of work done against the Lorentz force we estimate that the potential temperature gradient may increase by as much as

$$\delta G_p \sim \bar{B}^2 \bar{T} / (4\pi \bar{p} g d^2) = [\bar{B}^2 / (4\pi \bar{p} \mathcal{R} d)] (H_p / d) \quad (6)$$

where $\mathcal{R}$ is the gas constant. When $d \sim H_p$ this is comparable with the estimate of δG_a obtained above. Thus the alteration of the adiabatic gradient is one of several competing mechanisms which modify convective transfer through the action of strong magnetic fields arising at the base of the convective zone. These mechanisms have comparable numerical significance and we focus on one to estimate the order of magnitude of the resultant luminosity variation.

The convective zone itself adjusts to mild changes within a few turnover times of the largest eddies, that is months, but the underlying radiative zone requires much longer. In 11 yr thermal diffusion affects only a thin skin at the top of the radiative zone, whose thickness is 1,000 km and whose heat capacity is far smaller than that of the convective zone. Fluctuations in the convective zone do eventually influence a deeper region having a heat capacity comparable with its own, but that process takes

* Permanent address: Department of Applied Mathematics and Theoretical Physics, University of Cambridge, Cambridge, CB3 9EW, UK.

10^5 yr (refs 15, 16). On the other hand, hydrostatic adjustments of the whole Sun occur within a few hours. We may therefore regard the convective zone as receiving a fixed flux from below, which it modulates during the 11-yr time scale of solar activity.

Whatever the precise dynamical details of such a modulation, its magnitude may be gauged from our estimate of δG_a . While the thermal state of the underlying radiative zone does not change much in the time of interest and the radiative flux from below remains nearly constant, the change in G_a must result in a change in convective flux in the deep convective zone as soon as the instability develops. This will be accompanied by a slight change in temperature at the base of the convective zone of the order of $\delta T \sim \delta G_a d \sim 0.05$ K. The thermal energy involved in this change is of magnitude

$$\delta U \sim C_p M_c d \delta G_a \quad (7)$$

where M_c is the mass of the convective zone. For the Sun, $M_c \sim 0.03 M_\odot$ and so $\delta U \sim 10^{39}$ erg. Locally the toroidal field rises from zero at reversal to its peak value in a time τ during which, by equations (5) and (7), the change in magnetic energy is comparable with δU . In the same period, there are slight changes in the Sun's structure, for even adiabatic processes allow exchanges among the potential, thermal and magnetic energy reservoirs.

These fluctuations affect the rate at which convection removes energy and conveys it to the solar surface. Such modifications of the convective flux reach the surface in a few months with consequent changes in the upper transition layer of the convective zone. Here, radiative transfer becomes the dominant heat transport mechanism. The energy carried up by convection is converted into local thermal energy and radiated into space, hence the temperature of this layer must be close to the effective temperature, T_e . We wish to estimate the amplitude of temperature variations in this layer that result from convective changes and to infer the magnitude of concomitant changes in solar luminosity.

Solar models, based on mixing length theory, indicate that the transition layer is quite thin: its depth is about one local scale height and its mass $m \sim 10^{-9} M_\odot$. Its thermal response time, $m C_p T_e / (4L)$, where L is the luminosity, is ~ 5 min. On this time scale this crucial layer adjusts itself to accommodate changes in the rate at which convection carries energy outward. The changes of interest here are of magnitude $\delta U / \tau$, and they are directly reflected in fluctuations in T_e and L . The former are given approximately by

$$\delta T_e \sim \delta U T_e / (4L\tau) \quad (8)$$

and, for $\tau \sim 6$ yr, this yields $\delta T_e \sim 2$ K. Then, from equations (6) and (7), we find that

$$\delta L \sim M_c \bar{B}^2 / (8\pi\bar{\rho}\tau) \quad (9)$$

which does not depend on the thickness, d , of the magnetic layer. This gives a luminosity variation, $\delta L \sim 10^{-3} L_\odot$.

As we do not have an independent estimate of the radius variation, we cannot be explicit about the phases of the various changes. These enter through the familiar relation

$$\delta L / L = 4 \delta T_e / T_e + 2 \delta R / R \quad (10)$$

In agreement with our estimates, a change of 0.1% in luminosity requires that $\delta T_e \approx 1.5$ K if R remains constant. The relevant measurements have been discussed elsewhere¹⁷: the best data indicate that T_e decreases⁸ by about 6 K from sunspot minimum to sunspot maximum and that R increases¹⁹ by about 0.015% in the same time. These observations together with equation (10) point to the value $\delta L / L \approx 4 \times 10^{-3}$, which agrees with the above estimate.

Magnetic corrections to G_a and G_p have therefore to be included in studies of variations of solar luminosity over the solar cycle. Although we cannot reliably predict the phases of such variations, application of the viral theorem²⁰, modified to allow for the fluctuations we are discussing here, suggests that an increase in luminosity accompanies a decrease in solar radius

and *a fortiori* an enhancement of effective temperature. Livingston's measurements show a decrease in surface temperature as the sunspot cycle approaches maximum¹⁸; hence the solar luminosity ought to decrease in the intervals preceding sunspot maxima.

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Seasonal variation of oriental sunspot sightings

D. M. Willis & M. G. Easterbrook*

Rutherford and Appleton Laboratories, Ditton Park, Slough SL3 9JX, UK

F. Richard Stephenson

Department of Geophysics, University of Liverpool, PO Box 147, Liverpool L69 3BX, UK

An important, though inexact, index of past solar activity^{1–3} is the frequency of unaided-eye sunspot sightings in the Orient^{1,2–7}. When the glare of the Sun is reduced by a natural atmospheric filter, a single sunspot or compact group of sunspots located within the central part of the solar disk can be seen with the unaided eye if its area exceeds 0.5% of the Sun's visible hemisphere⁸. The oriental sunspot records represent one of the few available sources of solar data before the invention of the telescope^{4,5}. The abundance of historical sunspot records from China and Korea must be attributed partly to the regular occurrence of atmospheric conditions suitable for the detection of sunspots^{4–6}. Considerably diminished solar brightness, caused by, for example, atmospheric haze, dust storms, severe atmospheric absorption (for example, near sunrise or sunset) or reflection off still waters, is necessary to render sunspots visible to the unaided eye. Indeed, many of the oriental sunspot records explicitly mention reduced solar brightness⁶ compatible with these conditions. We discuss here what information the dates of the oriental sunspot sightings provide on past atmospheric conditions in the Orient and hence on past solar activity.

* Present address: Laboratory for Planetary Atmospheres, Department of Physics and Astronomy, University College London, UK.

Figure 1 shows the number of sunspot sightings that have been recorded in each month of the year according to a catalogue of pre-telescopic sunspot sightings (139 in all)⁶ which is based on an independent search of official oriental dynastic histories. These sightings have been used to obtain the seasonal variation in the occurrence of atmospheric conditions favourable to the detection of sunspots with the unaided eye. Three sightings have been discarded because only the year of the sighting is known; a fourth has been omitted because it is the sole sighting recorded in Japan, where such phenomena seem to have attracted less attention.

The shaded part of the histogram in Fig. 1 shows the distribution of those dates (year, month and day) in the catalogue⁶ that are specified without reservation. The unshaded part includes 12 dates for which either the day or month is uncertain; in such cases the most plausible historical date is used. In addition, each of the intervals within which the dates of eight other sightings lie has been contracted (from ~60 to 30 days) after re-examining the historical records. In these latter cases, which also contribute to the unshaded part of the histogram, the median day has been adopted as the date of the sighting. The probability of obtaining the shaded histogram by chance is less than 1 in 25,000, according to the χ^2 test⁹ with the theoretically expected frequencies assumed to be proportional to the number of days in the month. If the unshaded part of the histogram is included, this probability is still smaller. Therefore the seasonal variation of oriental sunspot sightings is highly significant statistically and should not be affected appreciably by the sporadic nature of the sightings. A similar seasonal variation exists in the data before (70 sightings) and after (65 sightings) AD 1150, a convenient date that divides the data into two subsets containing approximately the same number of sightings. The seasonal variation also exists separately in the Chinese (103 sightings) and Korean (32 sightings) observations.

Figure 1 presents the composite seasonal variation of sunspot sightings arising from a combination of various atmospheric conditions occurring at different geographical locations. The probable places at which most of the oriental sunspot observations were made are shown in Fig. 2. Despite the diversity of locations, the main features of the seasonal variation can be explained in terms of known characteristics of the general climate of much of East Asia. Thus the marked spring maximum can be ascribed to the fact that dust storms and sand storms ('yellow winds') originating over central China occur most

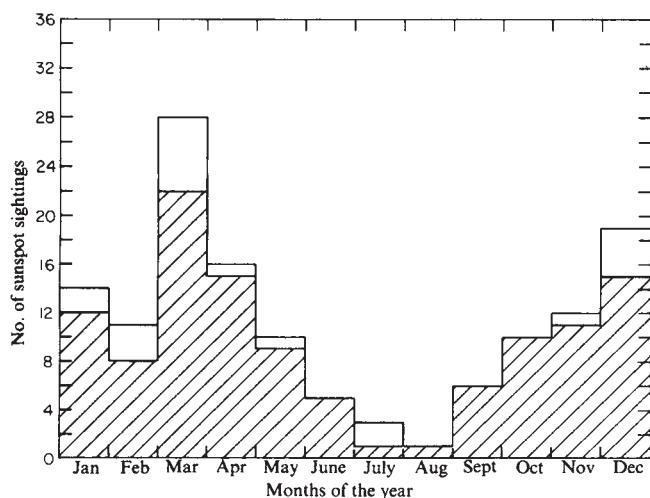


Fig. 1 Number of unaided-eye sunspot sightings recorded in each month of the year according to a catalogue of pre-telescopic sunspot sightings⁶. The date of the first sighting is 28 BC 10 May (Julian calendar) and that of the last sighting is AD 1604 25 October (Gregorian calendar). All dates have been corrected to the Gregorian calendar to allow for the gradual change in the dates of the seasons in the Julian calendar. The shaded part of the histogram refers exclusively to reliable dates, whereas the unshaded part includes dates for which the day or month is uncertain (see text).

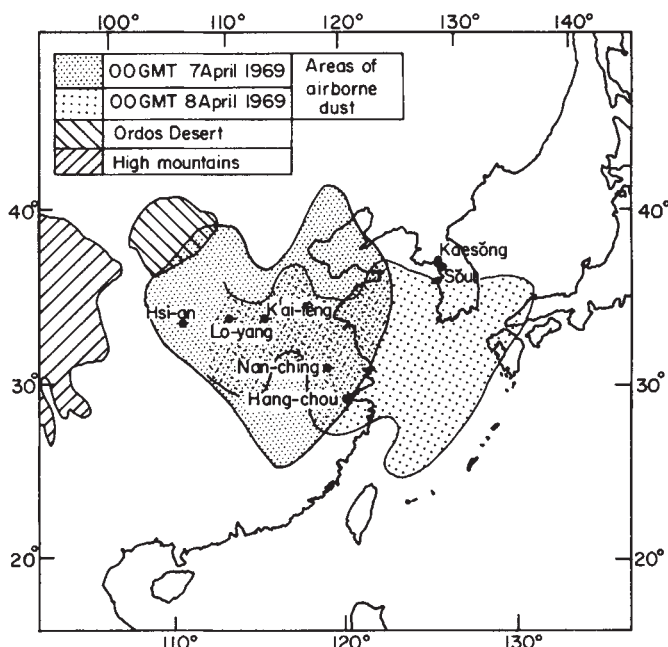


Fig. 2 The sites at which most of the ancient oriental sunspot observations were made. The areas affected by airborne dust or sand on two consecutive days during the large dust storm in April 1969 are shown for comparison. This storm was initiated by the passage of three successive cold fronts over the loesslands and Ordos Desert. The stippled areas define the regions within which meteorological stations reported blowing dust or sand: ESSA 9 satellite photographs confirm the large extent of this dust storm (after Ing¹²).

frequently during late winter and early spring, when aridity due to low winter rainfall and increased surface winds associated with cold fronts contribute to their occurrence¹⁰⁻¹³. The source of atmospheric dust and sand is the vast barren land comprising the Takla Makan, Gobi and Ordos Deserts and the Chinese loesslands¹². Dust and fine sand carried into the lower troposphere by strong winds can be transported down-wind several thousand kilometres^{12,13,15}. The resultant haze often obscures the horizon and causes deep red sunsets throughout China as far south as Hong Kong¹⁶. Airborne dust and fine sand over China and Korea acts as a natural filter that reduces the glare of the Sun, thus allowing fine detail to be perceived on the solar surface. Ho Peng Yoke¹⁵ suggested that such atmospheric dust would produce favourable viewing conditions at sunrise and sunset, thereby making direct observations of the Sun possible. Furthermore, those sunspot sightings for which the oriental records quote a duration of several days⁵ mostly occurred during winter or spring, which also supports the suggestion that dust storms produce favourable atmospheric viewing conditions.

Colleagues who have travelled extensively in China have noted that a typical dust storm dims the Sun considerably but does not blot it out and may well provide the conditions necessary for observing fine detail on the solar surface with the unaided eye. During a typical dust storm the Sun appears a deep yellow colour. Several oriental sunspot records specifically mention that "the Sun was orange and had no brilliance"⁶ and thus it seems likely that atmospheric dust in the lower troposphere was responsible for many of the oriental sunspot sightings.

An optically dense volcanic dust veil in the stratosphere would also dim and redden the Sun¹⁷, but the residence times for such volcanic dust (or 'ash') are too long for the seasonal variation shown in Fig. 1 to be attributed to stratospheric dust. Although long-lived changes in atmospheric viewing conditions, resulting from changing amounts of volcanic dust in the stratosphere, may possibly have contributed to long-term changes in the frequency of oriental sunspot sightings, we show below that transient changes of dust in the lower atmosphere can probably account

Table 1 Number of severe winters per century in China (after Lamb²²) and the corresponding number of oriental sunspot sightings (from Clark and Stephenson⁶)

Century (AD)	6	7	8	9	10	11	12	13	14	15	16	17	Average	s.d.*
Severe winters	19	11	9	19	11	16	24	25	35	10	14	21	17.8	7.7
Sunspot sightings	12	0	0	6†	3	5	32	13	32	1	1	12‡	9.8	11.4

The correlation coefficient, $r = 0.89$, is significant at the 0.1% level§

* Standard deviation.

† The single Japanese sighting (AD 851 22 December, Julian calendar) has been omitted, as in Fig. 1.

‡ As Clark and Stephenson's catalogue⁶ is confined to pre-telescopic sunspot sightings (sightings before AD 1611), the entry for the seventeenth century is based on the table presented by Cullen²⁵, which includes 12 Chinese sightings recorded in standard histories (*cheng shih*). If the 21 Chinese sightings found in local records (*fang chih*) are included, this number becomes 33. The correlation coefficient is then 0.83, but the significance level is unchanged.

§ According to Student's *t*-test⁹; the correction for autocorrelation is negligible.

for much of the variability in the number of sunspot sightings per century.

As an example, Fig. 2 shows the large area affected by airborne dust and sand for the dust storm generated over central China in early April 1969¹²; ESSA 9 satellite photographs have been combined with surface data from meteorological stations to track and identify the synoptic features of this dust storm. This particular storm was associated with the passage of three successive cold fronts over the loesslands and Ordos Desert. Such a large dust storm can obviously dim the Sun considerably and perhaps create propitious atmospheric conditions for viewing sunspots at all of the ancient observing sites. Smaller dust storms might produce the right atmospheric viewing conditions in more limited geographical regions.

The broad summer minimum shown in Fig. 1 can be attributed to greater cloudiness and precipitation during the rainy season¹³. The number of overcast days is highest in summer in both the Yellow River Basin (Hsi-an, Lo-yang and K'ai-fêng) and the Yangtze Basin (Nan-ching and Hang-chou)¹⁸; a similar situation prevails at the two observing sites in Korea (Kaesŏng and Sŏul)¹⁹. This explanation relies mainly on optically thick clouds obscuring the Sun and hence reducing the likelihood of sunspots being detected, particularly at sunrise or sunset, although optically thinner clouds or fog may occasionally favour the detection of sunspots. Heavy summer rainfall also washes dust out of the lower atmosphere, which results in maximum glare from the Sun on clear days. Owing to the greater cloudiness in summer^{18,19}, however, this 'clear sky' situation occurs on relatively few days per month. The small number of sunspot sightings recorded in July and August (see Fig. 1) can therefore be regarded as a summer base level determined by a combination of greater obscuration of the Sun on cloudy days and greater glare from the Sun on clear days, with the cloudy days outnumbering the clear days. The above discussion indicates that the detection of sunspots with the unaided eye depends sensitively on local atmospheric viewing conditions involving several meteorological variables. There are, however, considerable regional differences in climate within a geographical area as large as that shown in Fig. 2 and further research is required to clarify the seasonal variations of sunspot sightings in different localized regions.

Needham⁴ has suggested that the Chinese may have viewed the Sun through pieces of semi-transparent jade, mica or smoky rock crystal. Further, Chu Wên-hsin²⁰ has described a method of observing solar eclipses and sunspots by looking at the reflection of the Sun in a basin of water blackened with Chinese ink, and very similar methods of observing the reflection of the Sun are actually mentioned in ancient Chinese astrological literature²¹. However, there is no convincing evidence that efficient artificial aids were used to dim the Sun systematically; indeed, from the viewpoint of the ancient observers, the use of such optical aids might negate any astrological predictions. Also, although many more sunspot sightings would have been recorded if an efficient artificial aid had been used, the seasonal variation might then be expected to depend on the duration (hours) of bright sunshine^{18,19}. This inference is not substantiated by the seasonal variation shown in Fig. 1.

We conclude that atmospheric viewing conditions complicate the interpretation of the oriental sunspot sightings as an index of past solar activity. In particular, as dust storms in East Asia are associated with the passage of cold fronts over deserts and loesslands, more sunspot sightings should have been noted during cold winters and springs. Most of the oriental sunspot sightings were indeed recorded during those centuries for which the number of severe winters in China was above average²², as indicated in Table 1; this association seems to be meaningful whatever the cause of the climatic changes in China. As the detection of sunspots with the unaided eye depends significantly on local atmospheric viewing conditions, the number of sunspot sightings in the Orient should not be compared with the severity of winters in geographical areas remote from China (such as Europe). However, if the frequency of the oriental sunspot sightings is still regarded as an approximate measure of past solar activity, rather than just an indicator of favourable atmospheric viewing conditions, the correlation ($r = 0.89$) between severe winters and sunspot sightings presented in Table 1 conflicts with Eddy's claim² that cold climate coincides with low solar activity. It now seems more likely that the oriental sunspot records provide merely circumstantial evidence for excursions in solar activity²³ and that the radioactive isotope ¹⁴C, preserved in the annual growth rings of trees, should be used as the prime guide to past solar activity. Nevertheless, the association between severe winters and sunspot sightings may help to elucidate the discovery in Chinese provincial historical records of six sunspot sightings during the 'Maunder minimum'^{24,25}, as the seventeenth century was one of the coldest in China²².

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Frequency sensitivity of the deep-sea climatic record

Thomas J. Goreau

Center for Earth and Planetary Physics, Harvard University, Cambridge, Massachusetts 02138

Attempts to explain quasi-periodic glacial/interglacial transitions¹ in the Pleistocene ¹⁸O deep-sea stratigraphic record have focused on spectral analysis of palaeoclimatic data and comparisons with Milankovitch-type calculations of variations in isolation caused by periodicities of the Earth's orbit around the Sun²⁻⁶. Periods close to those of orbital eccentricity (100 kyr), obliquity (41 kyr), and precession (22 kyr) can be identified in deep-sea cores, but their relative strengths are very different from those of solar insolation and other peaks are also present. Mixing of sediment by deposit-feeding infauna significantly perturbs stratigraphic boundaries in deep-ocean sediments⁷. Although mixing models⁸⁻¹¹ have been proposed to describe quantitative effects of bioturbation no formulation of the effect of this process on the spectrum of the climatic record has been presented. Using the signal processing model¹¹, the suppression of recorded climatic variation over the past 700 kyr is estimated. In the frequency range where changes in the Earth's orbital path affect incident solar radiation the results support an appreciably larger role for astronomical 'forcing' of climate than previously estimated.

Mixing is treated here as a process which filters input information arriving at the surface of the sediments from the overlying water. The mixing function, operating on the input, is the spatial distribution of an impulse input to the sediment surface after some period of mixing. Mixing proceeds by a series of stochastic events in which organisms overturn, ingest and excrete sediment while in search of food. On a sedimentation time scale huge numbers of such events occur.

The model evaluates the evolution of the profile through numerical solution of equations like:

$$O_{\tau}(x) = F_{\tau}(x) \frac{I_{\tau}}{S_{\tau}} + \int_0^{L_{\tau}} F_{\tau}(x) O_{(\tau-\Delta\tau)}(x-x'') dx''$$

where x is the depth in the core at time τ ; x'' a dummy variable; $\Delta\tau$ the time integration step; L_{τ} the maximum mixing depth; S_{τ} the sedimentation rate; I the input time series; F the spatial mixing function; and O the distribution of the output in the

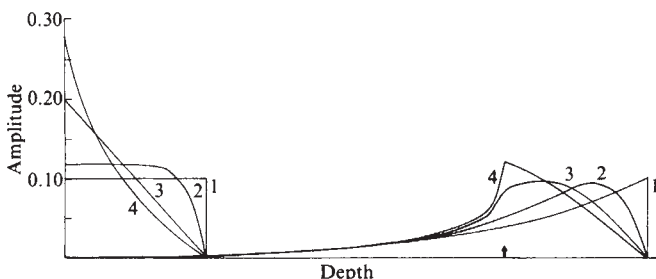


Fig. 1 Impulse response functions for mixing functions with the same maximum mixing depth but differing degrees of convexity and concavity. The curves at left show the 'instantaneous' (on a sedimentation time scale) response to a unit input at the top of the sediment, before burial. The curves at right show the output for unit inputs which have been delivered at the point shown by the arrow and subsequently mixed and buried. The impulse response function changes shape with increasing burial, but approaches asymptotic form after burial to about four mixing depths has occurred: only the initial and final shapes are shown. Amplitude reduction of unit inputs is sensitive to the shape of the mixing function near the surface, but almost insensitive to it after sufficient burial. Phase shifts between output and input are very sensitive to the shape of the mixing function. Deep-sea ash layer stratigraphy¹⁸ and microtektites¹⁹ give profiles of class 3, as do radionuclides such as ¹⁴C. The first are deeply buried and the last are near surface inputs. Simulations using ¹⁴C profiles show that they do not strongly constrain the shape of the mixing function.

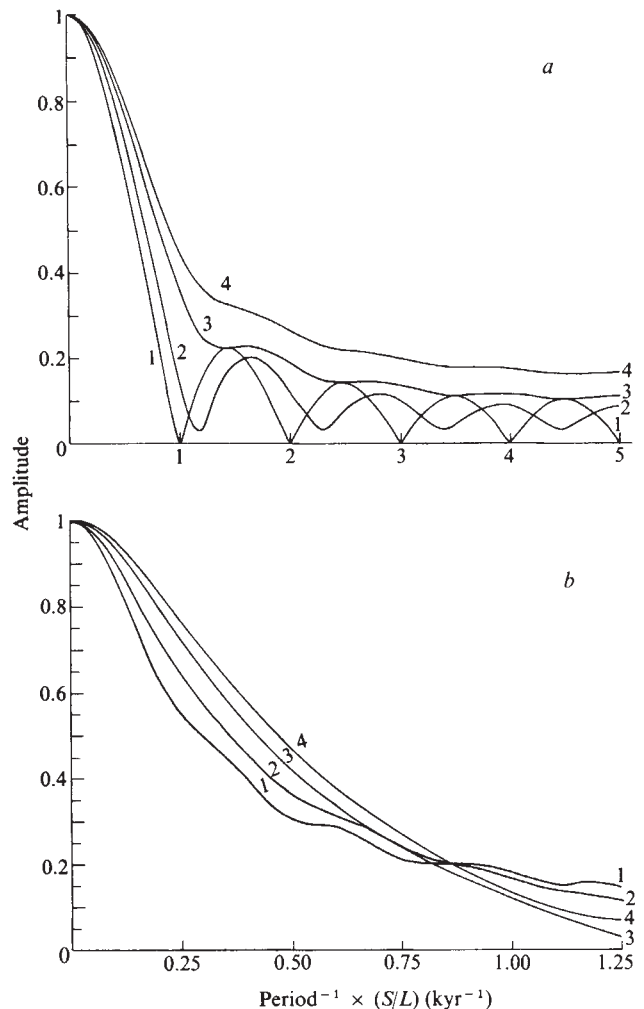


Fig. 2 *a*, The frequency response ('transfer') function for inputs to the surface before burial, calculated from the Fourier transform of the curves at the left of Fig. 1. Using curve 1 as a base for inverse filtering is numerically unstable because of the zeros in the curve. Frequency is scaled by the ratio of the sedimentation rate to the maximum mixing depth; a shape-dependent factor allows transformation to scaling by mean mixing depth, a convenient procedure when dealing with noisy data. *b*, Transfer functions following burial to asymptotic output shape under continuous mixing and burial. The curves of the right of Fig. 1 have been Fourier transformed as in *a* but note that the horizontal scales differ. The frequency band width of the bioturbation low-pass filter is about twice as wide before burial than after it so that high frequencies are somewhat better preserved at the very top of the core.

mixing zone. Mixing functions, as shown in Fig. 1, were chosen to simulate the range of bioturbation patterns inferred from stratigraphic, geochemical and benthic ecological studies, as well as functions used in simple models^{8,9}. The curves at the left of Fig. 1 show mixing functions of varying convexity and concavity, whereas those at right indicate the output following a long period of continuous mixing and burial following a pulse input. Profiles of deep-sea ash layers¹⁸ and microtektites¹⁹ resemble those of curve 3 (right) with maximum mixing depth of around 10 cm, whereas short-lived radionuclides have profiles peaked at the surface like Fig. 2*b* left, and a similar maximum depth. The impulse response displays transient behaviour until the input has been buried to a depth of $\sim 4L$, after which change in the shape of the output is negligible. This is convenient as deep downcore the output is a simple convolution integral of the input and asymptotic mixing function response¹¹, but it requires that treatment of recent inputs such as pollutants, ¹⁴C, U-Th series daughter products, and even the last glacial-interglacial transition use the full recursive model. Mixing models lacking 'memory' of previous outputs applied to the glacial meltwater 'spike'^{12,13} produce numerically unstable results which 'blow-up' the data (see, for example, ref. 14). The programme for recursive unmixing will be discussed elsewhere.

Figure 1 shows the impulse response in the spatial domain. In the frequency domain the mixing transfer function, which indicates the fraction of input at any frequency seen in the output, can be obtained by Fourier transform of the curves of Fig. 1. This has been done by calculating the coefficients of the Fourier Series expansions. Figure 2a shows the frequency transfer functions for inputs before burial, and Fig. 2b shows the transfer functions of inputs so deeply buried that their transients have relaxed. For an input of known power spectrum, the power spectrum of the output is derived by multiplying the input power spectrum by the square of the transfer function at each frequency¹⁵.

Climatic and other controls on local sedimentation rates introduce a non-linear filter that in the space domain is a measure of the uncertainty in the true age at each depth caused by variable sedimentation. This effect is particularly severe for high frequencies as small shifts in sedimentation alter apparent periodicities and suppress power. Present data on fluctuations of sedimentation rates are inadequate for quantitative treatment, although they limit the reliability of the record.

Curve *c* of Fig. 3 shows the power spectrum of Emiliani's¹⁶ composite ¹⁸O chronology for the past 730,000 yr (the late Pleistocene since the Brunhes–Matuyama magnetic reversal) computed from the periodogram determined by FFT⁴. Curve *b* shows the power spectrum of this climate record corrected for mixing by function 3 of Fig. 1 and appropriate values of *L* and *S*.

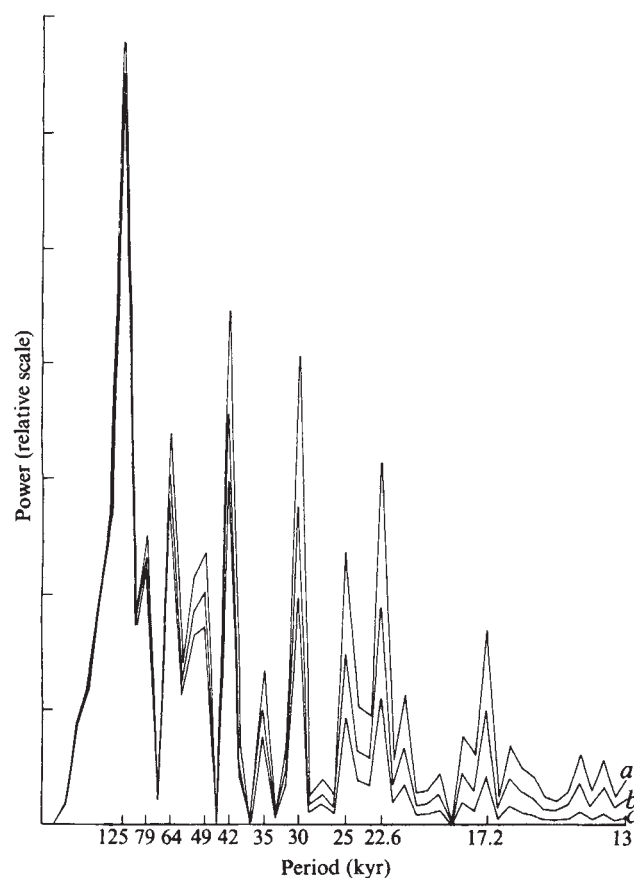


Fig. 3 Periodogram of observed climate variability of Emiliani's composite ¹⁸O curve for the past 730 kyr (ref. 16) computed by FFT⁴ (curve *c*). Curve *b* is the power spectrum uncorrected by curve 3 and curve *a* for uncorrecting by curve 1 (note that the asymptotic form has been used, thus avoiding the numerical instability caused when the surface response is used to deconvolve the input). Bioturbation decreases the power at the precession frequency by a factor of two or more with respect to the power of the fundamental frequency, dependent on the shape of the mixing function. The non-Milankovitch frequencies at 64, 30, and 17 kyr⁻¹ could be odd harmonics of the 125-kyr period produced by non-linear interactions but data are insufficient for statistical tests of this hypothesis without an underlying physical model (C. Wunsch, personal communication). The doublet at around 23 kyr results from splitting of the precession frequency by eccentricity modulation.

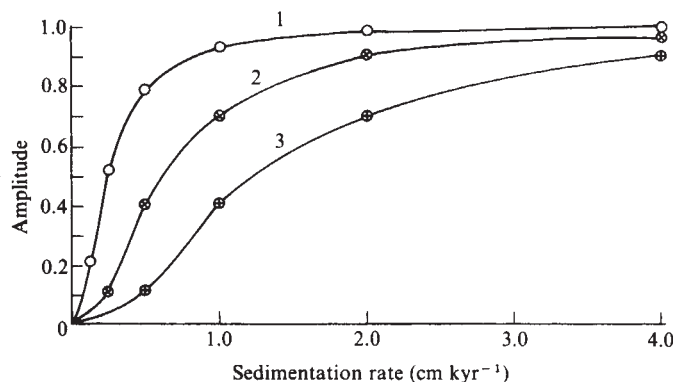


Fig. 4 Signal amplitude reduction at periodicities corresponding to those of the Earth's orbital eccentricity, obliquity, and precession for various sedimentation rates. These were calculated using curve 3 of Fig. 2b and *S/L* of 5 kyr⁻¹, a value consistent with the apparent sedimentation rates and mixing profiles of many deep-sea cores used in palaeoclimatic work. ○, 100 kyr; ⊗, 40 kyr; ⊕, 20 kyr.

Curve *a* shows the spectrum after correction for mixing pattern 1 of Fig. 1. Although such a pattern is atypical of deep-sea ash layers¹⁸, it is similar to that assumed in most mixing models. These calculations suggest that the observed power at frequencies corresponding to orbital precession has been suppressed by a factor of about two. The actual amount may be greater because of possible variability in sedimentation rate. No attempt was made to 'tune-up' the record by making features cohere with expected precession and obliquity peaks as done by some workers without markedly changing the spectra^{3,6}.

Figure 4 shows the effect of sedimentation rate on amplitude suppression at 20, 40, and 100 kyr periodicities for a mixing curve of type 3 and a mixing depth of 10 cm. Increased depth of mixing shifts the curves to the right (greater attenuation of input), and reduced depth shifts them to the left. Analogous curves for attenuation and phase shift for mixing functions of differing shapes and *S/L* ratios are readily computed. Events of less than 10-kyr duration (such as the last glacial–interglacial transition) are strongly attenuated in all but very rapidly accreting cores. These results support Erez's suggestion that differences in glacial–interglacial ¹⁸O amplitudes observed in cores from different oceans are greater than would be expected from mixing alone and are likely due to dissolution effects²⁰ or growth rate-dependent biological kinetic isotope fractionation during calcium carbonate deposition^{21,22} superimposed on ice-cap volume and local temperature signals.

Energy-balance models have been developed to explain climate changes^{23,24}. However, uncertainties in parametrizing atmospheric and oceanic heat transport, cloudiness, precipitation, albedo, and ice-cap growth as functions of insolation highlight the alternative approach: that of extracting as much information as possible from the historical record to estimate empirically climatic responses^{3-6,16}. It has recently been suggested⁵ that the observed climate spectrum is 'red' (power varying as the inverse square of frequency) in accordance with a climate model in which stochastic forcing at much higher frequency drives the lower frequencies seen in the deep-sea record²⁵. When bioturbation is taken into account this interpretation is weakened: the astronomical frequencies explain a larger portion of the observed variance (as the coherence with insolation is proportional to responses at 20 and 40 kyr), and the spectrum is no longer 'red', dropping off as approximately the $-1/2$ power of frequency.

Deriving climate transfer functions for the astronomical theory of climate is made difficult by the narrow bandwidth of the forcing frequencies. At the high frequency end bioturbation limits the response, and at low frequencies it is uncertain whether the 100-kyr period is a sharply peaked or a non-linear response to eccentricity, an artefact of short data length, or

generated by another process^{3,4,23,24}. A linear climate model would require long response times: crustal rebound time scales²⁶ are too short, but some highly parameter-sensitive models have generated appropriate time constants for polar ice-cap growth^{23,24,27}. The two processes are probably coupled²⁸. Whatever mechanisms regulate climate on these time scales, detailed understanding of bioturbation is needed to estimate response time scales to external and endogenous forcing from the deep-sea record. This record contains more information than is apparent once the rules for its encoding are understood. Further work on particulate and chemical tracers of mixing²⁹⁻³³ is needed to quantify the effects of bioturbation. Recent work on deep-sea ash layer stratigraphy^{29,30} will further constrain the mixing function's spatiotemporal patterns in the deep sea and allow improved understanding of climate change.

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Dolomite formation in the seawater-freshwater interface

M. Magaritz & L. Goldenberg

Isotope Department, Weizmann Institute of Science, Rehovot, Israel

U. Kafri & A. Arad

Geological Survey of Israel, Jerusalem, Israel

The 'dolomite problem' long defied solution by geologists largely because it could not be resolved in experiments or by comparative studies with Recent sediments. Most of the known Pleistocene dolomites are from evaporative environments, whereas most of the ancient ones occur in rocks which show no evidence of intertidal or supratidal evaporative conditions. A possible environment for Recent formation of dolomites is the mixing zone between freshwater and seawater. We consider here dolomite formation in the interface mixing zone between freshwater and seawater and suggest that this type of dolomite formation is much more common than was previously indicated.

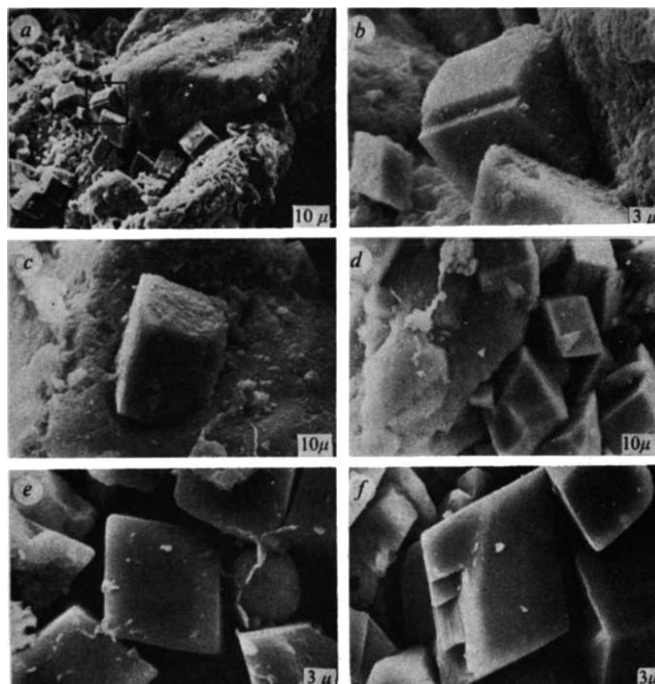


Fig. 1 *a*, Protodolomite rhombs cementing calcareous sandstone. Inset square is enlarged in *b*. *b, c*, Protodolomite rhombs attached to the surface of a quartz grain showing euhedral growth into the void. Imperfection of the euhedral form is found at the contact with the quartz grain. *d*, Protodolomite rhombs and calcite scalenohedron attached to bioclasts. Neither crystal shows evidence of replacement. *e*, Protodolomite cement and an unaltered coccolith. *f*, Subhedral protodolomite crystal.

Pleistocene to Recent dolomitization in hypersaline, evaporative environments has been reported by several authors¹⁻⁴. In addition, dolomitization and precipitation of dolomite has been noted from freshwater karst systems⁵. Recent dolomite formation in brackish waters of the mixing zone between freshwater and either hypersaline or seawater has been postulated and described⁶⁻⁸, particularly from carbonate coastal aquifers of the Caribbean region⁸⁻¹⁰.

It seems that the latter phenomenon is more abundant than was previously recognized, little attention having been given to this process taking place in the diffusion zone between freshwater and seawater in coastal aquifers. This is probably partly related to the fact that most wells in coastal aquifers did not penetrate the diffusion zone.

Fortunately, the Mediterranean coastal aquifer of Israel is densely drilled and has been well sampled and documented to depths below the freshwater-seawater interface. This Quaternary coastal aquifer consists mainly of alternating calcareous sandstones (eolianites), claystones and siltstones^{11,12}. The eolianites consist mainly of detrital quartz grains, transported from the Nile by longshore currents, together with 10% calcareous skeletal fragments. During exposure to meteoric water the bioclasts were recrystallized to coarse low Mg-calcite and calcitic cement was formed in the vadose zone^{13,14}.

Two groups of brackish water springs along the Coastal Plain of Israel, in the Zevulun Plain and southern Mt Carmel, were interpreted as mixtures of fresh aquifer water with intruding seawater^{15,16}. Carbonate concretions which were precipitated from these brackish waters in the upper soil sequence were found to consist of magnesian calcites and some authigenic dolomite¹⁷. The ¹⁴C age determination of the concretions from the Zevulun Plain was 3,200 ± 1,730 yr BP. (The age determination was carried out on magnesium-bearing calcite nodules which contain some protodolomite in the inner parts. The lack of

available material meant that the gas for the counting had to be diluted, a process which increased the uncertainty of the age.)

Dolomite has been found in cores from the central and southern coastal aquifer of Israel, forming at the freshwater-seawater interface. Two horizons of protodolomite were detected in the cores, the upper one coinciding with the diffusion zone of the present-day freshwater-seawater interface and the lower one at a deeper level¹⁸. Note that the protodolomite occurrences were found only in these two specific horizons and not throughout the sequence.

Protodolomite which forms up to 90% of the total carbonate (sample 3K 108-109) has been studied using scanning electron microscope (SEM) (Fig. 1). Crystal size reaches 50 μm (Fig. 1c). Samples with a low protodolomite content are typically composed of smaller (1-15 μm) crystals. Some protodolomite rhombs form a cement around detrital grains (Fig. 1a), being attached to the grain at one point and showing euhedral growth into the intergranular void (Fig. 1b, c). This results in subhedral crystals (Fig. 1f). The protodolomite was formed in voids and is not pseudomorphing pre-existing CaCO_3 . It precipitated directly from the solution and the sources of the Ca^{2+} and part of the CO_3^{2-} could be from dissolution of CaCO_3 somewhere in the aquifer. This can be seen in Fig. 1a, b where unaltered carbonate bioclasts are cemented by protodolomite. The crystals resemble closely the limpid protodolomites reported from schizohaline environments^{8,19}.

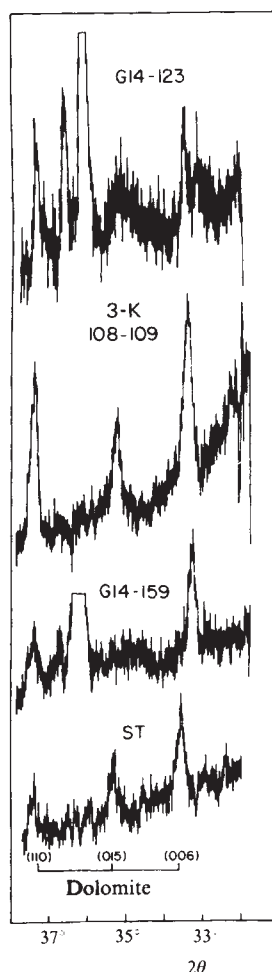


Fig. 2 X-ray diffraction traces showing variation in dolomite reflections in the studied samples compared to a standard ideal dolomite (ST). The ordering (015) reflection is broad and less intense than the non-ordering (110) peak. The 36.2° and 36.7° 2θ peaks are those of calcite and quartz.

All $\text{CaMg}(\text{CO}_3)_2$ minerals discussed here can be defined by X-ray diffraction and EDAX analyses as protodolomites²⁰. The outer surfaces of the crystals are deficient with regard to Mg^{2+} compared with ideal dolomites. The EDAX analysis shows a $\text{Ca}^{2+}/\text{Mg}^{2+}$ ratio between 6 and 7 (peak height) similar to some Florida limpid protodolomites¹⁹, whereas this ratio in a normal dolomite is around 4. In addition, the X-ray diffraction (112) peak is shifted to the lower 2θ values typical of protodolomites with excess Ca^{2+} .

The degree of ordering of the protodolomites was determined by measuring the (015) ordering peak height and comparing it to another peak not directly associated with these alternating cation planes²¹. In the samples discussed the (015) ordering peak is either small (sample 3K 108-109) or almost non-existent (sample G 14-159) compared to the (110) non-ordering peak. The basal reflection (006) is slightly shifted compared to standard dolomite (Fig. 2).

The $\delta^{18}\text{O}$ and $\delta^{13}\text{C}$ values obtained for sample 3K 108-109 are $-1.3 \pm 0.1\%$ (ref. 22) and $-9.6 \pm 0.05\%$ respectively. These are similar to the results from Jamaican protodolomites precipitated from a mixture of seawater and CO_2 oversaturated groundwater⁷. The $\delta^{13}\text{C}$ values of these samples are similar to those obtained for the low Mg-calcite cement (-9 to -11%) formed from meteoric water in the Israel Pleistocene coastal aquifer¹⁴. The $\delta^{13}\text{C}$ values of the dissolved carbonate in the aquifer near the coastline range between -10 and -12% and ^{14}C concentration range between 55 and 60 PMC (A. Kaufmann personal communication) indicating a mixed source for the dissolved carbonate (soil CO_2 and carbonate mineral dissolution). The $\delta^{13}\text{C}$ values of the protodolomite is -9.6% indicating an equilibrium between the protodolomite and the dissolved carbonate in the groundwater. The $\delta^{18}\text{O}$ of the protodolomite, however, differs by 4‰ from the calcitic cement. The same difference of 3-4‰ was experimentally obtained and has been attributed previously to isotopic fractionation between calcite and protodolomite²³.

The basic requirement for the dolomite depositing solution is that $[\text{Mg}^{2+}]/[\text{Ca}^{2+}] > 1$, and this ratio is reached at a chlorinity level¹⁷ of $1,000 \text{ mg l}^{-1}$. As the cementing calcite and the protodolomite of the coastal aquifer discussed here are in isotopic equilibrium (having the same source of CO_3^{2-}) the solution must constitute more than 95% of the fresh groundwater. Dolomite formation is apparently proceeding in the subsurface of the Israeli Coastal Plain in a calcareous sandstone aquifer where the Mg^{2+} is mainly derived from seawater and where an isotopic equilibrium between calcite and protodolomite is reached.

We can now demonstrate that in addition to Recent dolomitization of an evaporative (supratidal) type, a non-evaporative dolomite precipitation is also currently occurring. This process seems to be more widespread than considered before, as it exists in a wide region where the mixing zone between fresh groundwater and intruding seawater occurs. In most of the known coastal aquifers, the contact between the two water bodies does not appear as a sharp interface, but as a wide diffusion zone occupied by brackish waters.

The existence of more than one level of dolomite precipitation in the sequence arises from the fact that the interface is not necessarily stationary. It moves vertically, shrinks and spreads with corresponding eustatic changes of sea level and seasonal fluctuations of the water table. Therefore, dolomites below the present-day interface might indicate 'palaeo-interfaces' or 'palaeo-diffusion zones' which were stationary during certain periods when lower sea levels prevailed, and are in agreement with the Quaternary history of the Mediterranean sea levels.

This continuous process of dolomite precipitation in the diffusion zone is responsible for the reported reduction of $[\text{Mg}^{2+}]/[\text{Ca}^{2+}]$ ratio²⁴ and it might also affect the reduction of permeability of some of the coastal aquifers by reducing their pore space.

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^{243,244}Cm in Columbia River sediments

T. M. Beasley & L. A. Ball

School of Oceanography, Oregon State University,
Marine Science Center, Newport, Oregon 97365

Holm and Persson¹ have described the presence in lichens of ²⁴⁴Cm resulting from the testing of nuclear devices. As the retention of transuranic radionuclides by lichens differs markedly as discussed by the above authors, integrated inventories of ²⁴⁴Cm (mCi km⁻²) resulting from fallout were not computed, nor were samples earlier than 1961–62 analysed to determine possible curium isotope production during testing in 1952–58. We have now measured ^{243,244}Cm as well as ²³⁸Pu, ^{239,240}Pu and ²⁴¹Am in sediments from the Columbia River which show that (1) ^{243,244}Cm was produced in weapons tests before 1961, and (2) the total ^{243,244}Cm activity produced during testing was 1–3% that of ^{239,240}Pu. Even though the Columbia River received large amounts of radioactivity as a consequence of the operation of plutonium production reactors on the Hanford Reservation², we believe that the ^{243,244}Cm reported here is entirely derived from fallout and that our ability to detect these isotopes in river sediment is due to the concentrating effect of erosional processes on land which mobilize material containing ^{243,244}Cm (and other transuranic radionuclides) to the river, where they are subsequently sedimented.

The data presented here were derived from a sediment core raised from McNary Reservoir in August 1977. McNary Reservoir is located approximately 100 km downstream from the Hanford Reservation in Washington State and is formed by waters held by McNary Dam. It represents the first major accumulation site of fine-grained sedimentary material transported by the Columbia River after its confluence with the Snake, Yakima and Walla Walla rivers. On retrieval of the core, the sediment was extruded, sectioned and 0.5 cm trimmed from the outer rim to remove down-trained material. Each section was subsequently weighed, dried, homogenized and an aliquot dissolved in HNO₃ and HF. Calibrated amounts of ²⁴²Pu and ²⁴³Am were added before processing to quantify the recovery of Pu, Am and Cm isotopes. We have determined that our chemical procedures do not fractionate Am from Cm and therefore ²⁴³Am can be used as a tracer for Cm isotopes. Our radiochemical procedures have been tested in blind intercalibration exercises³ with excellent results. Except for the mass spectrometry data discussed below, all activities were determined by α spectrometry using silicon surface barrier detectors. Because

the energies of the α particles emitted by ²⁴³Cm and ²⁴⁴Cm (5.79 and 5.82 MeV, respectively) are nearly identical, the relative percentages of the two isotopes cannot be distinguished by present-day α -spectrometry techniques. This becomes moderately troublesome where decay corrections are required owing to the different half lives of the two isotopes (²⁴³Cm, $t_{1/2}$ = 28 yr; ²⁴⁴Cm, $t_{1/2}$ = 17.9 yr). Therefore, the data reported here and those of Holm and Persson must be viewed with this constraint.

We will consider in turn our contention that curium isotopes were produced in weapons tests before 1961, that the curium isotopes we measure are derived from fallout rather than from the Hanford reactors and by inference, must be accumulating in environments similar to McNary Reservoir, and finally that by assuming identical mobilization from land for both Pu and Cm isotopes the total amount of curium isotopes deposited globally from weapons testing is 3–10 kCi.

Table 1 lists the activity concentrations of the transuranic radionuclides measured over the core length. The Pu and Am activities represent values at the time of collection; column 5 shows the curium isotope activities at the time of measurement (mid-1979) and column 6 the year represented by the respective sediment horizons. The sedimentation rate at this coring site (~ 1.8 cm yr⁻¹) was determined from the position of the ^{239,240}Pu subsurface maximum (identical to the subsurface maximum for ¹³⁷Cs) and the abrupt change in the ²³⁸Pu/^{239,240}Pu ratio at 21 cm indicating the arrival of SNAP-9A ²³⁸Pu in the Northern Hemisphere in mid-1966 (ref. 4). Our attempts to describe geochronologies using ²¹⁰Pb have been unsuccessful in McNary Reservoir because of the rapid sedimentation rates occurring there (2–5 cm yr⁻¹), the varying within-core textural qualities of the sediments, and the low activities of ²¹⁰Pb present (1–2 d.p.m. per g dry wt). All these conditions preclude the use of ²¹⁰Pb for establishing reliable sedimentation rates⁵.

From the data of Table 1, we conclude that ^{243,244}Cm was produced in devices tested before 1961; unfortunately, low levels of activity present at depths >40 cm in our core prevented us fixing earlier production dates precisely. For the following reasons we do not believe the ^{243,244}Cm we measured in this core was derived from the operation of the various single-pass plutonium production reactors located at the Hanford complex which were in operation in 1944–71. First, from mass spectrometry measurements (T. M. Beasley, L. A. Ball and B. A. Blakesley, unpublished data), we have determined that the mean ²⁴⁰Pu/²⁴²Pu ratio observed in several cores raised from McNary Reservoir is 41 ± 9 . Krey *et al.*⁶ have reported a mean ²⁴⁰Pu/²⁴²Pu ratio of 40 ± 10 for global fallout on land. We consider that the agreement between these data sets suggests that release of Pu isotopes with mass numbers greater than 239 from the reactors did not occur and therefore the presence of their decay products (Am isotopes) could not have served as parent isotopes of ^{243,244}Cm through neutron capture and subsequent β decay.

Mass spectrometry results have also shown that approximately 25% of the ^{239,240}Pu radioactivity in McNary Reservoir sediments is reactor-derived. The mean ²⁴¹Am/^{239,240}Pu ratio from the data of Table 1, when corrected for this contribution, is 0.29. This is only slightly higher than the measured range of 0.22–0.25 characteristic of fallout-derived material as measured in the early to mid-1970s (refs 6, 7). Our values are somewhat higher due to the further decay through 1979 of ²⁴¹Pu ($t_{1/2}$ = 14.9 yr), the parent isotope of ²⁴¹Am. As the most probable event leading to direct introduction of ^{243,244}Cm to the river would have been fuel element rupture in the reactors, such events would most certainly have been accompanied by release of Pu isotopes and ²⁴¹Am. Because no excess ²⁴⁰Pu appears in the sediments and because our ²⁴¹Am/^{239,240}Pu ratios are very near those of fallout, we think direct release of curium isotopes by the reactors unlikely.

To calculate integrated inventories of transuranics at the coring site (activity per unit area) the activity per g dry wt of sediment and the measured bulk dry density (g cm⁻³) of each

Table 1 Activity concentrations (d.p.m. per kg dry wt) of transuranic radionuclides in Columbia River sediments

Depth (cm)	^{238}Pu	$^{239,240}\text{Pu}$	^{241}Am	$^{243,244}\text{Cm}$	Year deposited
0-2	4.2±0.6	71±3	15.4±1.1	ND	
2-4	2.3±0.4	37±2	6.9±0.8	ND	
4-6	2.2±0.4	37±2	8.5±1.0	ND	
8-9	2.1±0.4	39±2	8.2±1.3	ND	
9-10	2.2±0.3	41±2	6.1±0.7	ND	
10-11	3.2±0.4	106±7	13.1±1.2	ND	
13-14	2.9±0.4	43±2	13.1±1.3	1.2±0.5	1970
14-15	3.0±0.4	51±4	9.4±0.6	0.6±0.2	
15-16	2.2±0.6	57±5	9.1±0.6	0.9±0.3	1969
16-17	3.4±0.5	73±3	16.3±0.6	0.8±0.2	
19-20	3.5±0.5	86±4	21.1±0.7	1.4±0.2	1967
22-23	3.6±0.5	105±4	28.7±2.2	1.4±0.2	
23-24	4.2±0.5	124±4	29.9±1.9	3.1±0.6	Mid-1964
24-25	3.9±0.5	117±4	29.3±2.1	2.9±0.9	
25-26	4.5±0.7	173±7	35.8±2.2	1.9±0.9	Mid-1963
26-27	4.0±0.6	147±6	32.4±1.8	1.6±0.4	
34-35	3.1±0.4	106±4	28.3±1.0	1.7±0.3	Mid-1958
39-40	1.9±0.4	79±3	18.1±1.2	0.8±0.2	1956
53-54	ND	4±0.7	ND	ND	
56-57	ND	0.16±0.08	ND	ND	

ND, not detectable. Errors represent 1 s.d. of the measurement determined from propagated counting errors.

section were plotted against depth through the core. The product of the mean activity per g dry wt and the mean bulk dry density over each 2.5 cm of core length was then summed to give the total activity per unit area. Errors through the summation process were propagated by standard techniques (1σ). We calculate integrated inventories of 19.4 ± 0.5 , 4.3 ± 0.5 and 0.21 ± 0.07 mCi km⁻² for $^{239,240}\text{Pu}$, ^{241}Am and $^{243,244}\text{Cm}$, respectively, as of May 1979. Subtracting a 25% reactor contribution from the total $^{239,240}\text{Pu}$ activity gives a fallout-integrated inventory for these isotopes of 14.5 mCi km⁻², which is seven times the cumulative deposition that has been measured in undisturbed soils at 45° N latitude⁸. We have observed even higher cumulative deposits within the reservoir (>50 mCi km⁻²), where both sedimentation rate and sediment texture lead to more rapid accumulation of fine-grained material. Clearly, erosional processes have mobilized the transuranic radionuclides from the landscape where they are then sedimented to the reservoir floor. Although the description of such processes is not novel, the degree of enrichment seen here for transuranic radionuclides is, we think, instructive.

Assuming $^{239,240}\text{Pu}$ and $^{243,244}\text{Cm}$ are mobilized by erosional processes to the same degree (a reasonable assumption based on our Am/Pu ratios measured), the amount of $^{243,244}\text{Cm}$ in our core was 1.4% that of the fallout $^{239,240}\text{Pu}$ in May 1979; considering the errors involved with individual inventory estimates, this percentage could be as low as 0.9% or as high as 1.9%. Were all the curium isotope activity due to ^{244}Cm (as was assumed by Holm and Persson for lichen), we calculate a decay-corrected total deposition of ^{244}Cm over the 14-40-cm interval of our core of 0.34 ± 0.12 mCi km⁻², a value representing 2.3% of the total $^{239,240}\text{Pu}$ deposited (range 1.5-3.2%). As the half life of ^{243}Cm is significantly longer than that of ^{244}Cm , this latter calculation represents the maximum curium activity which could be present, and therefore we estimate that curium isotope deposition from fallout lies between 1 and 3% that of $^{239,240}\text{Pu}$. As the $^{239,240}\text{Pu}$ inventory for global fallout has been reported at some 325 kCi (ref. 9), between 3 and 10 kCi of curium isotope activity has resulted from weapons testing.

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Lower Cretaceous sediment from the East Antarctic continental shelf

Eugene W. Domack*, William W. Fairchild† & John B. Anderson*

* Department of Geology, Rice University, Houston, Texas 77001

† International Biostratigraphers Inc., 5933 Bellaire Blvd, Suite 108, Houston, Texas 77036

Except for a few localities along the Antarctic Peninsula, on Alexander and Livingston Islands (Fig. 1), there are no known outcrops of Cretaceous sedimentary rocks in Antarctica¹. Thus, little is known concerning the palaeogeography and palaeoclimatology of Antarctica during the Cretaceous period. During Operation Deep Freeze 1979 to the George V-Adelie coast East Antarctica, a 40-cm long piston core, DF 79-38, was recovered which contains abundant palynomorphs of early Cretaceous age. Core 38 is an organic-rich siltstone believed to have been deposited in a non-marine setting. It represents the oldest known sediment recovered from the Antarctic continental margin and the first evidence for Cretaceous strata in East Antarctica. In addition, its exact location and sedimentary characteristics suggest an important correlation with similar sediments from the South Australian continental shelf.

Based on structural considerations, the probable existence of Cretaceous strata in Marie Byrd Land, West Antarctica (Fig. 1), was suggested by geologists of the New Zealand Antarctic Survey of 1958-59 (ref. 2). Cretaceous deposits are widespread on the South Australian continental shelf^{3,4} and Kemp⁵ discovered 'reworked' Lower Cretaceous palynomorphs in surficial glacial marine sediments collected off the West Ice Shelf, East Antarctica (Fig. 1). From this she suggests the local presence of sedimentary rock sequences of approximate Albian age. Reworked Cretaceous foraminifera have also been reported in Quaternary deposits from Taylor Valley, Transantarctic Mountains⁶.

Core 38 penetrated a mono-lithological breccia which contains clasts of grey, organic-rich siltstone. Small flecks of carbonized organic matter and wispy laminations are conspicuous. Clasts are 0.5-4 cm in size and are highly angular. The breccia is clast-supported except for the upper 5 cm which contains a muddy matrix. Core 38 was taken at a depth of 1,407 m along the seaward flank of the George V Basin (67°44'S, 146°51'E Fig. 2). Based on available core coverage this unit is probably overlain by a relatively thick section of glacial and glacial marine sediments, some of late Quaternary age⁷ (Fig. 2).

Five samples from the depths 8-10, 15-18, 24-29, 30-33, and 37-40 cm in Operation Deep Freeze Core DF 79-38 were processed for palynological study using standard maceration techniques. Each sample yielded abundant palynomorphs, comprising mainly spores with smaller amounts of saccate pollen. No significant differences were noted from top to bottom of the interval studied, suggesting that the entire interval was deposited in a single environment. The absence of marine microplankton (dinoflagellates) in these assemblages indicates deposition in a non-marine environment. Twenty-four species, listed in Table 1, were identified from core 38 samples, but the assemblages are so diverse and well preserved that many additional forms could undoubtedly be recognized with detailed study.

Table 1 Palynomorphs recognized from DF 79-38

<i>Aequitriradites spinulosus</i> (Cookson and Dettmann) Cookson and Dettmann 1961
<i>Alisporites grandis</i> (Cookson) Dettmann 1963
<i>Ceratospores equalis</i> Cookson and Dettmann 1958
* <i>Cicatricosisporites australiensis</i> (Cookson) Potonie 1956
<i>Classopollis classoides</i> Pflug emend Pocock and Jansonius 1961
* <i>Crybelosporites stylosus</i> (?) Dettman 1963
<i>Cyathidites australis</i> Couper 1953
* <i>Cyclosporites hughesi</i> Cookson and Dettmann 1959
<i>Foraminisporis wonthaggiensis</i> (Cookson and Dettman) Dettmann 1963
<i>Ischyosporites punctatus</i> Cookson and Dettman 1958
<i>Klukisporites scaberis</i> (Cookson and Dettmann) Dettmann 1963
<i>Stoverisporites lunaris</i> (Cookson and Dettmann) Norvick and Burger 1975
<i>Leptolepidites verrucatus</i> Couper 1953
<i>Lycopodiumsporites austroclavatis</i> (Cookson) Potonie 1956
<i>Lycopodiumsporites circolumenus</i> Cookson and Dettman 1958
<i>Microcachrydites antarcticus</i> Cookson 1947
* <i>Murospora florida</i> (?) (Balme) Pocock 1961
<i>Cepulina truncata</i> (Cookson) Scultz 1967
<i>Osmundacidites wellmanii</i> Couper 1953
* <i>Pilosporites notensis</i> Cookson and Dettmann 1958
<i>Schizoporis parvus</i> Cookson and Dettmann 1959
<i>Schizoporis reticulatus</i> Cookson and Dettmann 1959
? <i>Trilobosporites trioreticulosus</i> Cookson and Dettmann 1958, maybe
<i>T. purverulentus</i> (Verbitskaya) Dettmann 1963
<i>Velosporites triquetrus</i> (Lantz) Dettmann 1963

* The most stratigraphically useful species according to Dettmann and Playford⁸.

An extensive review of Cretaceous palynology in Australia was presented by Dettmann and Playford⁸. They recognized four distinct spore-pollen zones as well as two subzones within the early Cretaceous. The oldest, *Crybelosporites stylosus* zone, was considered to be Neocomian; the *Cyclosporites hughesi* subzone as Neocomian to Aptian; the *Crybelosporites striatus* subzone as Aptian; and the youngest, *Coptospora paradoxa* zone, as Aptian to Albian. These zones have since been stratigraphically used in palynological studies in southern and eastern Australia. Most recently, Burger⁹ found the zonation scheme of Dettmann and Playford⁸ a workable one for Lower Cretaceous sediments of the Surat Basin in East Central Australia. In addition, Burger was able to correlate with faunal evidence and fix the age of his *Osmundacidites dubius* zone as Aptian⁹. Burger equates this zone with the middle and upper part of the *C. hughesi* subzone of Dettmann and Playford^{8,9}.

Many species identified in core 38 range throughout the Lower Cretaceous in Australia and elsewhere, and are thus of little value for precise age determinations. Five species in Table 1 are considered important stratigraphic indicators by Dettmann and Playford⁸, most of these were also recognized by Burger⁹. A single specimen questionably identified as *C. stylosus* was noted in the sample from 37–40 cm. According to Dettmann and Playford this species is restricted to the *C. stylosus* zone, although Burger lists it as ranging up into the *O. dubius* zone⁹. *Murospora florida* and *C. hughesi* are found both in the *C. stylosus* zone and the *C. hughesi* subzone. *Pilosporites notensis* is also present in the *C. hughesi* subzone but ranges up into the *C. striatus* subzone as well. Comparison of the species identified in core 38 samples with those discussed by Dettmann and Playford⁸ suggests that the assemblage present correlates fairly well with the *C. hughesi* subzone or the *O. dubius* zone of Burger⁹, and that it is of early Cretaceous, Aptian age.

The continental margins of South Australia and East Antarctica, between 90° E and 150° E, represent classic passive-rifted margins^{10,12}. The best morphological fit of the two continents before break-up seems to be that of Sproll and Dietz¹⁰; see also Weissel and others¹³. This fit is consistent with subsequent reconstructions of Australia–Antarctica with New Zealand, the Campbell Plateau, and other continental crust regions of the South-West Pacific margin of Gondwanaland¹⁴. According to

their reconstruction, the continental shelf off the George V Coast, where core 38 was taken, lay directly south of the city of Adelaide and west of the Otway Basin, Australia (Fig. 3). Recent seismic profiling and exploratory drilling on the continental shelf of Australia has revealed a series of east–west trending normal faults^{4,15}. Faulting occurred in the latest Cretaceous to earliest Tertiary, was associated with early rifting between the two continents, and produced a horst and graben structure typical of rifted margins¹⁶. Down-dropped blocks contain up to 2,500 m of Lower Cretaceous age sediments which are fluvial-lacustrine in origin¹⁵. Transitional marine to marine sediments do not occur below the uppermost Cretaceous–Lower Tertiary stratigraphic interval, which unconformably overlies the Lower Cretaceous section.

The outer continental margin of East Antarctica, north of the shelf break, contains marginal rift structures similar to those developed along the southern Australian margin¹⁷. The George V Basin, of maximum depth¹⁸ of 1,591 m, is one of several deep, inner shelf depressions which form a more or less continuous basin parallel to the coast of East Antarctica between 90 and 150° E (ref. 19). This feature is thought to represent a graben structure bounded by faults of Tertiary age¹⁹. Vanney and Johnson²⁰, in a more detailed analysis of the orientation of these basins, have also proposed a tectonic origin for these features.

The presence of Lower Cretaceous non-marine strata in the George V Basin implies a sedimentological and tectonic setting similar to that of the South Australian continental shelf during the Cretaceous. However, subsequent sedimentary and palaeo-environmental conditions of the two regions have differed considerably, particularly with the development of a continental ice sheet on Antarctica, which is generally believed to have originated during the late Oligocene to mid-Miocene^{5,21,22}. Unfortunately, early Tertiary sedimentary sequences from Antarctica are as limited as those of the Cretaceous period. With the discovery of early Cretaceous sediments from the East Antarctic continental shelf it seems likely that early Tertiary strata may be found in a similar setting. Future work should concentrate on coring of these marginal basins where a more complete sedimentary sequence may help clarify the palaeo-climatological setting of Antarctica.

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Marine influence in the Triassic 'uplands'

John E. A. Marshall

Department of Geology, The University, Newcastle upon Tyne
NE1 7RU, UK

David I. Whiteside

Department of Geology, The University, Bristol BS8 1TR, UK

Several authors¹⁻⁴ have concluded that Mesozoic reptiles found in some fissures of the Bristol area belonged to a distinctive 'upland' fauna. We describe here a new fissure deposit recently found in the Carboniferous limestone near Thornbury which contains characteristic 'upland' reptiles associated with an assemblage of Rhaetian marine phytoplankton and land-derived palynomorphs. The marginal marine location of deposition deduced from the palynomorphs conflicts with the separation of late Triassic reptiles into 'upland' and 'lowland' regimens.

The new fissure is located 16 km north of Bristol in Tytherington quarry (ST 660 890). In 1975, before the level was

Table 1 Palynomorph assemblage from the Tytherington fissure

	% of major elements (>1%)
<i>Alisporites thomasi</i> (Couper) Nilsson 1958	2
<i>Cingulizonates rhaeticus</i> (Rheinhardt) Schultz 1967	
<i>Cingulitrites infrapunctus</i> (Schulz) Morbey 1975	
<i>Corollina torosus</i> (Klaus) Cornet & Traverse 1975	9
<i>Corollina zwolinskai</i> Lund 1977	
<i>Krauselisporites reissingeri</i> (Harris) Morbey 1975	
<i>Limboisporites lundbladii</i> Nilsson 1958	
<i>Lunatosporites rhaeticus</i> (Schultz) Warrington 1974	
<i>Ovalipollis pseudoalatus</i> (Thiergart) Schuurman 1975	25
<i>Rhaetipollis germanicus</i> Schulz 1967	3
<i>Riccisporites tuberculatus</i> Lundblad 1964	18
<i>Triancoraesporites ancorae</i> (Rheinhardt) Schulz 1967	
<i>Tsugaepollenites pseudomassulae</i> (Madler) Morbey 1975	
<i>Vesicaspora fuscus</i> (Pautsch) Morbey 1975	4
<i>Cymatiosphaera polypartita</i> Morbey 1975	2
<i>Dapcodinium priscum</i> Evitt 1961	
<i>Michystridium</i> spp.	
<i>Rhaetogonyaulax rhaetica</i> (Sarjeant) Fisher and Van Helden 1979	19

flooded, several tons of fissure material were recovered and are now held in the University of Bristol.

The fissure material, composed of red and green marls, breccias and conglomerate, contains various terrestrial reptile bones, marine reptile fossils being entirely absent. Archosaur bones, such as those of *Thecodontosaurus*, are disarticulated and often in preferred orientation, but skeletal elements showing little sign of wear are frequently found associated; this suggests that transport has been very limited. Lepidosaur bones (including *Clevosaurus*), a few of which are water worn, occur widely distributed in the sediments. The reptiles include forms often termed as the Triassic 'upland' fauna¹⁻⁴ in contrast to the 'lowland' taxa with rhynchosaurs which are absent in the Bristol fissures.

The palynological assemblage was recovered from a grey siltstone. UV fluorescence studies⁵ show that the palynomorphs were deposited in organic-rich laminae and have not been introduced in discrete reworked clasts. Furthermore, the assemblage itself is very well preserved. These findings therefore preclude any suggestion that the palynomorphs are derived from a Rhaetian deposit and reworked into the fissure in later times.

The palynomorph-bearing siltstone lies in a small pocket and although it borders an archosaur bone layer it has not itself yielded animal fossils. However, the nature of the enclosing fissure sediments (breccia, conglomerate and detritus-laden marls) and their chaotic bedform suggest a sedimentary episode of short duration. The most likely hypothesis is a cavern roof collapse with restricted pockets of silt forming in the spaces between the fallen rocks where the current flow would have been markedly reduced. In view of this situation and the finding of reptile remains from the same species in the marls enclosing the breccia and siltstone, we conclude that the palynomorphs and reptile bones were deposited penecontemporaneously.

The palynological assemblage (Table 1) has been compared with others of the European Rhaetian⁶⁻⁸ and unequivocally equates with the *Rhaetipollis germanicus* zone (phase 3). A comparison with other UK assemblages⁹⁻¹¹ shows that the sporomorph elements and proportions are consistent with a Rhaetian age from the Westbury or possibly lower Cotham Beds. Furthermore, the high percentage of the dinocyst *Rhaetogonyaulax rhaetica* is also consistent with this placing as there is a population acme for this species in the other British Rhaetian outcrops.

A further comparison with local British Rhaetian outcrops shows that the fissure palynomorphs have no distinctive elements which could be ascribed to an ecologically separate

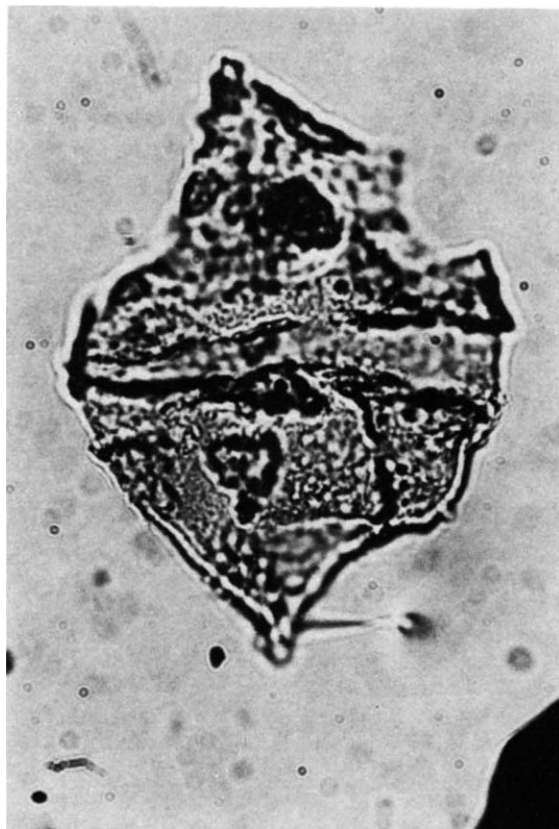


Fig. 1 The dinocyst *Rhaetogonyaulax rhaetica* (Sarjeant) Fisher and Van Helden, 1979 from the Tytherington fissure, $\times 1,000$, transmitted light

Table 2 Kerogen typing

Phytoplankton	4.5%	Total marine derived 51.5%
Amorphous kerogen	47%	
Wood	31%	
Cuticle	2.5%	Total terrestrial derived 48.5%
Spores/pollen	15%	

'upland' flora. The presence of acritarchs and dinocysts, well known from the nearby Rhaetian sections, indicate a marine input into the fissure. By analogy with the findings of Wall in some British Jurassic strata¹², the occurrence of the acritarch *Michystridium* sp. (as opposed to *Veryhacium*) suggests a near-shore (brackish?) environment and explains the absence of a stenohaline marine fauna. A morphological typing (Table 2) of the total kerogen shows that nearly half is amorphous organic material. Amorphous organic material is usually considered to have formed diagenetically¹³ in marine conditions from algal biopolymers, through a monomer stage to give a condensed geopolymer. The high proportion of amorphous organic material in combination with the consanguineous Rhaetian dinocysts indicate a strong marine component and preclude any suggestion that the marine input was blown into an upland fissure by surface winds.

We postulate that the fissure was partly submerged under the transgressing Rhaetian sea. There is a patch of 'Rhaetic' (probably of the upper Westbury Beds) exposed 1 km south of the quarry and at a present-day altitude of over 20 m above the fossil-bearing cavern. This strengthens the suggestion that the lower fissure levels were flooded by saline waters; these would have brought in the organic-walled phytoplankton and provided the precursors for the formation of the amorphous organic material. At the same time, terrestrial vertebrates living around the entrance of the fissure and land-derived sporomorphs (showing no anomalous 'upland' elements) either fell or were washed into the cavern and were deposited with the marine palynomorphs.

This reconstruction of the environment exposes the anomaly of well preserved late Triassic 'upland' reptiles in a marginal marine deposit. Also, in the past, the difficulty of accurately dating 'upland' reptiles has meant that some 'lowland' taxa used for the comparison may not have been contemporaneous (for example, the last known record of a rhynchosaur is mid-Norian¹⁴ whereas the Tytherington upland reptiles are Rhaetian). In view of these findings we suggest that the loose term 'upland' cannot prudently be applied to distinguish these fissure reptiles from those of other late Triassic sediments.

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The first fossil cephalopod statoliths to be described from Europe

M.R. Clarke, Linda Maddock & E. Steurbaut

Marine Biological Association, Citadel Hill, Plymouth PL1 2PB, UK
Laboratorium Voor Paleontologie, Institute Gent, Belgium

Statoliths of cephalopods are small, hard calcareous stones which lie within the cartilaginous skulls of octopods, sepioids and teuthoids¹. Fossil statoliths, clearly belonging to genera which are alive today, have previously been described from 11 Cenozoic deposits spanning from the Eocene to the Pleistocene in North America²⁻⁵. Such statoliths are of particular interest because they provide a means of studying the evolution of living cephalopod groups which have no calcareous shells, including the cosmopolitan and numerous teuthoids and octopods. Here, the first cephalopod statoliths to be recognized in European deposits are described and identified as *Loligo* sp. They are compared with the North American fossil *Loligo* species and statoliths removed from the two living *Loligo* species of Europe.

Cephalopod statoliths were found in two Burdigalian outcrops of the Southern Aquitanian Basin (early Miocene: age 20-16 Myr). Blue-greyish fine sand was sampled at the hamlets of Paillon, Poyartin (French geological map 1/50,000; XIV-43, Dax: coordinates $x = 342.100$, $y = 158.420$) and Jean Tic, Saubrigues (XIII-43, Saint-Vincent-de-Tyrosse: coordinates $x = 305.550$, $y = 152.050$), and each sample of 750 kg only yielded three statoliths. The deposits occurring at Paillon are considered to be synchronous with the Burdigalian stratotype deposits of northern Aquitaine, whereas those from Jean Tic,

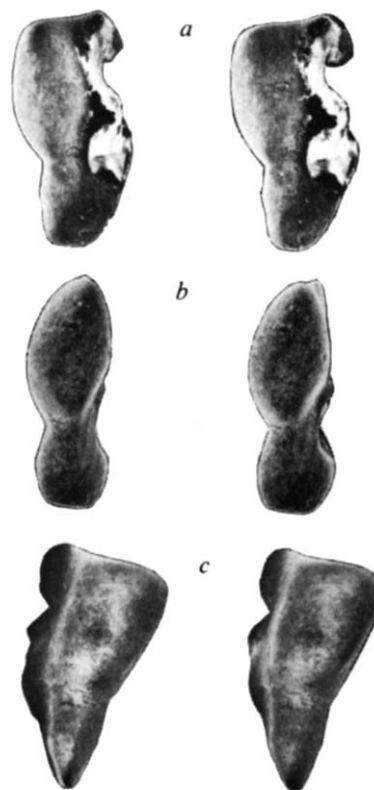


Fig. 1 a-c, Three stereo pairs of a fossil *Loligo* statolith (BM(NH) No. C46952) from a Miocene deposit in Europe photographed with a scanning electron microscope. These should be copied and suitably separated for obtaining a three-dimensional effect with a stereo viewer. Length of statolith = 1.75 mm.

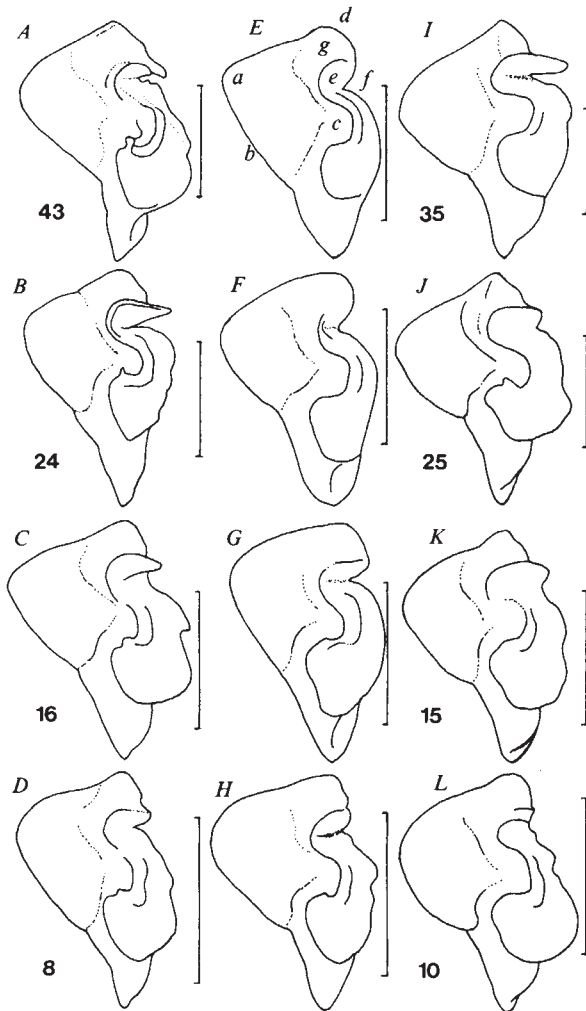


Fig. 2 Anterior views of *Loligo* statoliths. A–D, *L. vulgaris*; E–H, Miocene fossil statoliths from Europe (BM(NH) Nos. C46953–6). I–L, *L. forbesi*. Statoliths are shown as the same length to emphasize variation in form. Scales indicate 1 mm. The dorsal mantle length of the squids from which the statoliths were removed are indicated to the nearest centimetre. Drawings of statoliths F–H were reversed because they were the opposite side (left) to the others. Note that E and F are nearly the same length as C, G and K.

generally known as 'the Marls of Saubrigues', are certainly younger (? Upper or Post Burdigalian) and formed at greater depths⁶.

The overall shape of these six statoliths is closely similar to statoliths removed from living *Loligo* species and clearly different from statoliths of cephalopods in other families. In particular, the lateral dome is broadest and rather pointed at its dorsal end (Figs 1, 2a), the lateral dome is not divided into superior and inferior lobes (Fig. 2b) and is oval from the lateral side and the spur is prominent and almost square (Fig. 2c). Other notable features are that the dorsal dome extends dorsally further than the lateral dome (Fig. 2d), the dorsal indentation (Fig. 2e) is smaller than the ventral indentation being both shorter and narrower, there is a distinct medial fissure (Fig. 2f) and the anterior surface of the dorsal dome is flat but not markedly deflected anteriorly (Fig. 2g). Although previous work shows that certain of these features distinguish between several *Loligo* species examined elsewhere and statolith shape varies between individuals and with growth (Fig. 2), when these variations are taken into account there are no other obvious features which distinguish between the fossil and the two living species of Europe *Loligo vulgaris* Lamarck 1799 and *Loligo forbesi* Steenstrup 1856. The North American fossil species are more easily distinguished from each other³.

Comparison of these European fossils with North American fossil species shows that the European are closest in general shape to *Loligo mississippiensis* Clarke & Fitch 1979 from the Lower–Middle Oligocene deposit at Vicksburg, Mississippi³. They differ, however, from this species in having a less sharply pointed lateral dome which is more rounded anteriorly and ventro-laterally and has a deeper medial fissure.

To examine the species relationships further, nine dimensions of all the statoliths were used in a multiple discriminant analysis⁴. The measurements were defined previously^{1,3,4} and are termed total length (tl), rostral length (rl), lateral dome length (ld), ventro-lateral length (vl), dorso-lateral length (dl), dorsal dome length (dd), dorsal-tip-to-spur length (dts), maximum width (w) and thickness (th). This analysis maximizes the differences between the species on the basis of combining all nine dimensions and extracting axes in decreasing order of importance. Thus, the first and second axes used here account for the greatest proportion of the total variance and give the best separation between the species.

The six European fossil statoliths fall into two size groups of three, corresponding to the two sites from which they were collected, and as the *L. vulgaris* and *L. forbesi* statoliths also both fall into two such size groups the analysis was run with nine groups, six for these three species and three American fossil species. The first two axes extracted accounted for 94% of the total variation between groups and were both significant at $P=0.001$. All the scores on these two axes were standardized and the means and standard deviations of each of the nine groups are plotted (Fig. 3A).

Although there was some variation in the standard deviations between groups, the dispersions of the original variance-covariance matrices were not so different as to invalidate the discriminant analysis. The direction and magnitude of the effect

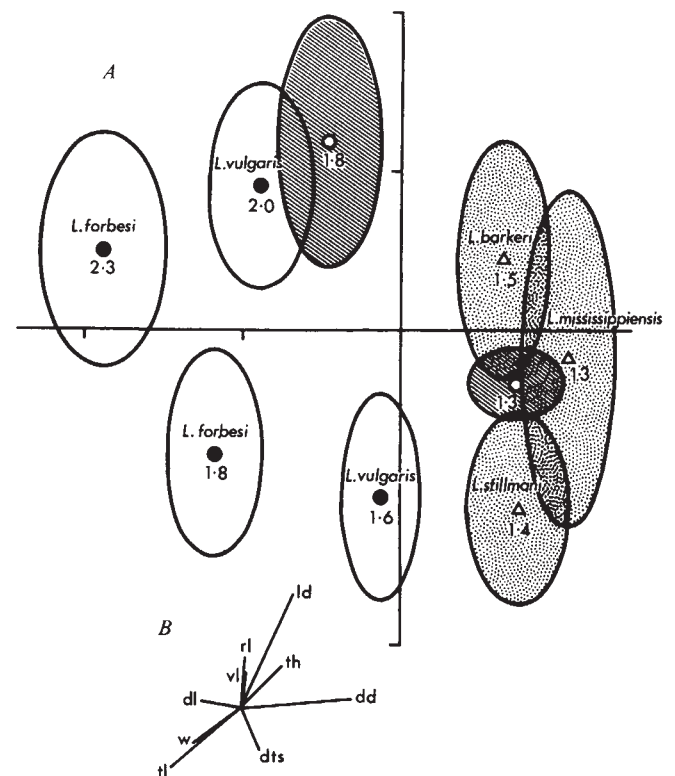


Fig. 3 A, Results of the multiple discriminant analysis of statoliths of three North American fossil species (stippled), one European fossil species (hatched) and two European living species of *Loligo*. The mean position of each species (or group) is plotted on the first two discriminant axes, with ellipses representing 1 s.d. for each group on each axis. The mean total length (in mm) of statoliths is given for each group. B, The relative weightings of the nine measurements on the axes. The measurements are identified as described in the text.

of each of the nine measurements on the separation of the species on the two axes are shown in Fig. 3B.

The spatial relationships between the groups of large and small statoliths of *L. forbesi*, *L. vulgaris* and the European fossil are very similar despite the fact that the fossil statoliths are smallest. This may suggest that the fossil species grew to a smaller adult than the living species. These fossils are approximately the same size as statoliths from *L. forbesi* and *L. vulgaris* individuals, with dorsal mantle lengths of 8.3–15.8 cm (Fig. 2). As the size of statoliths in the Loliginidae is closely correlated with the dorsal mantle length of the animals⁵ it is likely that the fossil statoliths came from animals having mantle lengths of approximately 8–16 cm.

When the scores for all individual statoliths were plotted on the first two axes there was no overlap between *L. forbesi* and any of the fossils, whereas the larger *L. vulgaris* overlapped with the larger European fossils. The smaller *L. vulgaris* overlapped with *Loligo barkeri* and *Loligo stillmani* and the smaller European fossils overlapped with all three American fossils.

Thus, although differences between the species are rather too subtle to make identification possible between individual statoliths of *L. forbesi* and *L. vulgaris* or *L. vulgaris* and the large European fossils, discriminant analysis shows that there is a definite trend in form from the American fossils through the

European Miocene fossil and *L. vulgaris* to *L. forbesi* (Fig. 3). Although the difference between the two size groups of the European fossil shown in Fig. 3 is probably a function of size, it might indicate a difference in species, because they come from different sites whose fauna show some differences. For example, the fish otoliths assemblage of Paillon, in which the smallest statoliths were found, differs from that of Jean Tic in having no mesopelagic representatives and in having many genera which are restricted today to the Indian Ocean. In addition, the association of Jean Tic contains some typical species recorded only from younger strata (ref. 6 and unpublished data). Such features are due to the difference in depth of deposition, but also reflect slight differences in age.

These data show that *L. vulgaris* or a closely similar species lived in European seas during the early Miocene. These seas were rather shallow (~30 m; the deepest parts, the canyon in which the Marls of Saubrigues were deposited, certainly did not exceed 300 m) and the climatic conditions were similar to those on the Atlantic coast of southern Morocco today.

Thus, the form of the statolith has remained virtually unchanged during a period of 20 Myr.

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Best shape for nature reserves

Margaret Game

Nature Conservancy Council, 19/20 Belgrave Square,
London SW1X 8PY, UK

The ideas of classical island biogeography¹ have been used^{2–6} to derive rules for the optimal design strategy for nature reserves. For example, Diamond³ states that, given limited (financial) resources, it is better to purchase a few large reserves rather than many small ones of equal total area; and that reserves should be as close to one another as possible. The validity of some of these rules has, however, been questioned^{7–10}; for example it has recently been shown^{9,10} that several small reserves may contain more species than a single one of equivalent area. These rules have nevertheless been accepted uncritically by others, including the IUCN¹¹. Here, I examine Diamond's rule³ that reserves should be as round as possible and conclude that in certain circumstances the optimal shape may be other than circular. There is no *a priori* reason for believing that these circumstances are unrealistic, and I know of no observational evidence to suggest whether they are found in nature or not. I also reason that the rule that reserves should be as close to each other as possible is inconsistent with the statement that they should be circular.

According to the tenets of island biogeography, the number of species inhabiting islands or isolated habitat patches (such as many nature reserves) is governed by the rates of extinction and immigration¹; when these balance each other on average, the number of species is in equilibrium. If the extinction rate is higher than that of immigration, the island is supersaturated and the number of species will decline or 'relax' towards equilibrium. The rate of relaxation or net loss of species can be reduced by decreasing the rate of extinction or by increasing that of immigration and the purpose of Diamond's rules is to achieve both of these. For example, he states³ that a large reserve is preferred to a smaller one because, amongst other things, it will have a lower extinction rate; and that reserves should be "as close to each other as possible . . . to increase the immigration rates between reserves".

If immigration into nature reserves is indeed an important factor maintaining the total number of species, then it is questionable whether reserves should preferably be circular as this requirement implies the reduction of extinctions without reference to any simultaneous effects on immigration rate. To many dispersing organisms the apparent size of an island, and therefore the probability of colonization, must be more a reflection of the island's linear dimensions perpendicular to the direction of travel, that is the apparent width and height, rather than the area (J. L. Harper, personal communication). Therefore, immigration rate will depend on shape, not just area, whether the organisms are dispersed by voluntary or involuntary means. Thus, whereas departure from circularity may adversely affect extinction rate, this may be ameliorated by an increase in the immigrant population. The optimal shape for a reserve will depend on the balance between these two factors.

To investigate this quantitatively, define the dimensionless parameter R by¹²

$$R = \frac{P}{2(\pi A)^{1/2}}$$

where p is the island perimeter and A its area. R is a simple measure of shape; for any given area A , R may vary from unity for a circular shape to infinity for an infinitely long and narrow one. R is 1.1 for a square and 5.7 for a hedge 100 m long by 1 m wide; the greater the departure from circularity, the greater is R .

Assuming that the effects of shape act independently of other influences, such as area and isolation, the average extinction and immigration rates, E and I , may be approximated by^{1,4,13}

$$E = SVf(R)$$

$$I = (P - S)Ug(R)$$

where $f(R)$ and $g(R)$ are functions describing the effects of shape on E and I , S is the number of species present on the island at the given time, P is the total number of species in the species pool available for colonization, and V and U express the effects of other factors such as area, isolation, habitat diversity and such. To simplify the analysis, I ask: what value of R maximizes the equilibrium number of species, S_0 ? An alternative but qualitatively similar question would have been: what

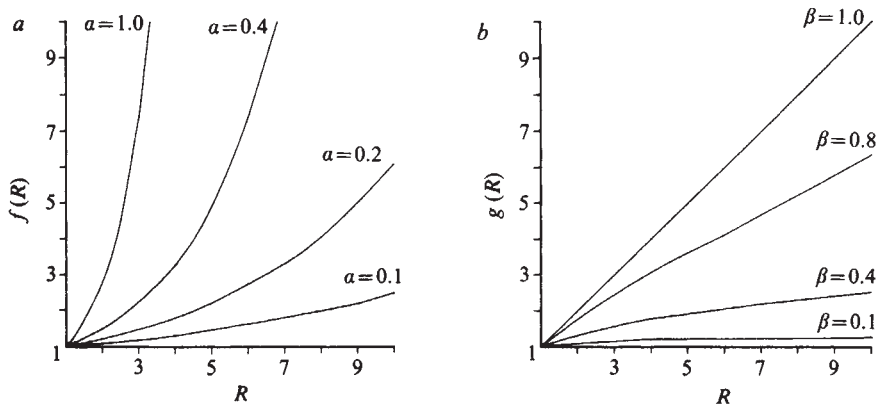


Fig. 1 a, Variation of $f(R) = C^{\alpha(R-1)}$ with α . b, Variation of $g(R) = R^{\beta}$ with β .

value of R minimizes the net extinction rate, $E - I$? At equilibrium, $I = E$, so that

$$S_0 = \frac{PUg(R)}{Vf(R) + Ug(R)}$$

and this is maximum at constant U and V when

$$f(R) \frac{\partial g(R)}{\partial R} = g(R) \frac{\partial f(R)}{\partial R} \quad (1)$$

I now ask what values $f(R)$ and $g(R)$ may reasonably be expected to take. It might be expected that the influence of shape on E is relatively slight when R is low (nearly circular reserves), but becomes severe at large R so that $f(R)$ would vary with R as shown in Fig. 1a. On the other hand, $g(R)$ might rise roughly linearly with R (which is proportional to perimeter length p) or level off towards some asymptotic value, as shown in Fig. 1b. Examples of functions with these general shapes, normalized to $f(1) = g(1) = 1$, are:

$$f(R) = \exp(\alpha(R-1)) \text{ with } \alpha \geq 0$$

$$g(R) = R^{\beta} \text{ with } 0 \leq \beta \leq 1$$

Substituting these functions in equation (1), the value of R which yields the highest value of S_0 is given by

$$R = \frac{\beta}{\alpha} \quad (2)$$

(It is easily verified that this represents a maximum rather than minimum in S_0 .) As $R \geq 1$, equation (2) only has a solution when $\alpha \leq \beta$.

The conclusion is: if extinction rate is not too dependent on shape (α is small) but immigration rate has a relatively stronger dependence on shape (β is not small), then the optimal shape for a reserve to maximize the equilibrium number of species is not circular. If these conditions are not met (that is, $\alpha \geq \beta$), so that a long boundary length affects the extinction rate more than the immigration rate, then the optimal shape is circular.

The above conclusion does not depend qualitatively on either the exact forms of f and g which were used or the equilibrium state being achieved. In general, if I depends relatively strongly on shape and E relatively weakly at least at low R , then there is no *a priori* reason to believe that a circular reserve will lead to the greatest number of species being conserved. However, this result does depend on immigration contributing significantly to the maintenance of the total number of species.

There are several ways in which reserves may come into existence and this will influence whether immigration or extinction processes predominate. Perhaps the commonest mode of creation is the preservation of a remnant habitat patch while surrounding habitat is removed or degraded. However, reserves have also been created *de novo* by establishing habitats on a site where they had been destroyed or damaged. When existing habitat is being preserved, species extinctions are likely to dominate immigration if the reserve is either heavily supersaturated or has become relatively isolated. Supersaturation

may occur through at least two processes. If habitat is destroyed, mobile creatures may concentrate in remaining patches. Alternatively, if the 'area-per se' hypothesis is correct^{1,14,15} and the number of species increases with area because greater populations of each species cause a decrease in extinction probabilities, then if habitat area is suddenly reduced the site may be supersaturated for a time depending on the rate of relaxation. It is not known whether many reserves in Britain are supersaturated in this sense; Terborgh² and Diamond¹⁶ believe that supersaturation occurs on some tropical islands and that it takes thousands of years for the avian fauna to relax to equilibrium, but this time scale is questioned by Abele and Connor⁸.

Isolation of suitable habitat affects the ability of species to colonize. Many reserves in Britain are relatively remote from each other, reducing the probability of inter-reserve transfer. However, although species in need of conservation are often sedentary¹⁷⁻¹⁹ this is not the case for migratory animals or for some plants such as orchids and bryophytes (M. O. Hill, personal communication). Therefore there are cases where immigration is an important factor even when long established habitat is involved. Immigration must certainly be of primary significance when new habitat is created. Note that where immigration is not an important factor, there is no need to locate reserves as close to each other as possible because the possibility of inter-reserve transfer of 'desirable' species is slight. Therefore Diamond's rules concerned with reducing mutual isolation of reserves are inconsistent with his statement that reserves should be as circular as possible: the first assumes that immigration is important and the second that it is not.

Finally, reserve shape may also affect other factors not explicitly accounted for in the above analysis. In particular, as J. L. Harper (personal communication) has pointed out, soil diversity and other habitat heterogeneities might be greater in long, linear reserves than in circular ones, and this might increase the number of species.

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Habitat selection maintains a deleterious allele in a heterogeneous environment

J. S. Jones & R. F. Probert

Department of Genetics and Biometry, University College London, 4 Stephenson Way, London NW1 2HE, UK

Little is known of how the large amount of genetic variation found in natural populations is maintained. Diversifying natural selection in a heterogeneous environment has great potential for promoting genetic polymorphism¹. If one allele is favoured in one microhabitat and the alternative allele in another (which may be separated from the first by space or time), the disruptive effects of selection can maintain both alleles in the population. The limiting conditions of niche size, selective intensity and gene flow which could in theory maintain polymorphism in this way have been widely discussed^{2,3}. This mechanism is much more effective in promoting genetic diversity if carriers of the alternative alleles are able to select the niche in which their fitness is greatest⁴. Here we explore the properties of a system in which two *Drosophila* eye colour alleles, known to show differences in fitness, are given the opportunity to select different microhabitats in a population cage. It seems that habitat selection in a heterogeneous environment can lead to the maintenance of polymorphism for an allele which is disadvantageous in either habitat when no opportunity for choice is available.

Eye colour mutants in *Drosophila* alter their carriers' response to light^{5,6}. Wild-type (red-eyed) flies are in general positively phototactic, but are repelled by very bright light⁷. Flies lacking red screening pigment in the eye are more sensitive to light and tend to choose habitats in which light intensity is lower⁸. Wild-type flies are effectively blind in dim light (which is blocked by their screening pigment) whereas white-eyed flies (which lack this pigment) move towards dim red light as it is within their preferred range of hue and intensity. They avoid the bright white light selected by the wild type. White-eyed *Drosophila melanogaster* are considerably less fit than the wild type in white light, and are rapidly eliminated from polymorphic populations in cages⁹.

Drosophila simulans (unlike *D. melanogaster*) mates less well in reduced light¹⁰. It is therefore possible that, in dim red light, white-eyed *D. simulans* might have a mating advantage over the wild type, as this is within their perceptual range but below that of the wild type. There is thus the opportunity to design a system in which lights of different hue and intensity cause flies of different genotype at the eye colour locus to select, because of their phototactic behaviour, an environment in which they are relatively fit. We describe here an experiment on the role of habitat choice and diversifying selection in a patchy environment on eye colour polymorphism in *D. simulans*.

Experimental populations were founded in cages of volume 8,750 cm³ each of which was divided into two by a vertical partition having at its upper edge a 20 × 2-mm slit through which flies could pass freely. In the five white light controls, both halves of the cages were illuminated from above by a bright white light produced by three 80-W 'daylight' fluorescent tubes. The light in the five red light controls resulted from the attenuation of this by 10 thicknesses of red filter material to give a dim red light in

both sections of the cage. In the five experimental cages, one half was illuminated by the bright white and one half by the dim red light. Each population was founded with 120 homozygous fertilized females from a wild-type strain, and the same number of fertilized female homozygotes from a white-eyed strain. The founding generation of flies was phased in over 3 weeks to reduce later population fluctuations. Cages were kept at 22–25°C and contained 200 ml of cornmeal–agar–molasses medium, one-third of which was replaced each week. Samples were taken by scoring the offspring of four sampling vials introduced to each side of the cage at 3-week intervals.

Figure 1 shows the course of gene frequency change. The frequency of the *w* allele drops rapidly in both white light and red light control cages; by week 30 its mean frequency in the white light controls is 0.01 and in the red light controls 0.06. However, although this allele is deleterious in both environments when no habitat choice is available, in the heterogeneous environment of the experimental cages the polymorphism is maintained for at least 30 weeks; the mean frequency of *w* in the patchy cages after this period is 0.32. Habitat selection (which may lead to an apportionment of resources between flies of different genotype) is maintaining polymorphism for an allele which in a uniform environment is lost from the population.

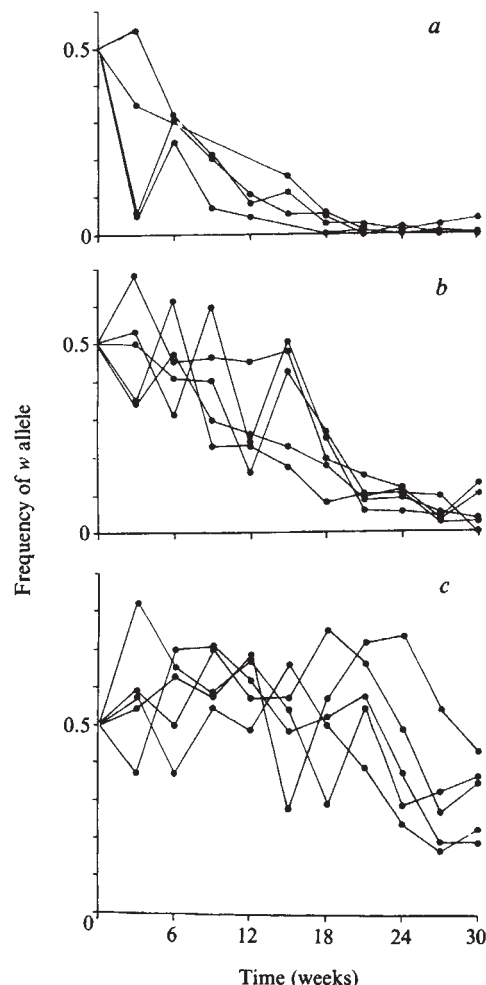


Fig. 1 Changes in the frequency of the *w* allele in *D. simulans* in: *a*, white light controls, *b*, red light controls and *c*, heterogeneous environments. One of the white light controls became extinct early in the experiment. Allele frequencies at this sex-linked locus were estimated using the weighted mean frequency in each sex. Although this does not provide an exact measure of allele frequency (as Hardy–Weinberg conditions do not hold) it is the most efficient estimate available.

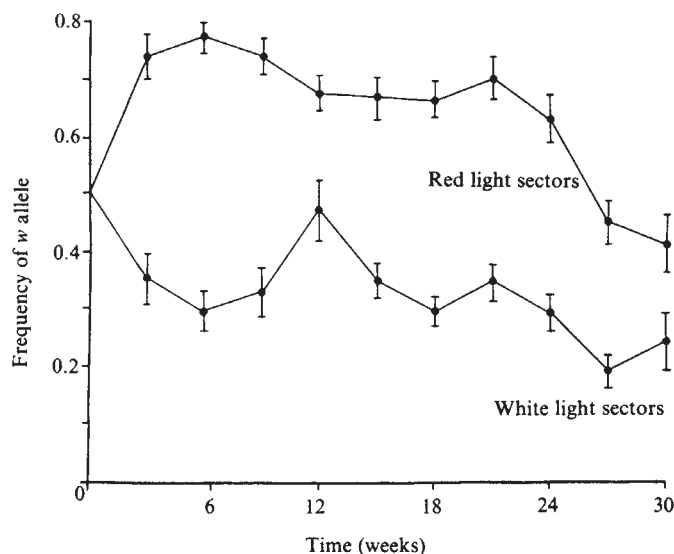


Fig. 2 Divergence in the frequency of the *w* allele between the red light and white light sectors of the experimental cages, summed over all cages.

There is considerable divergence in allele frequency between the two halves of the experimental cages (Fig. 2). To assess gene flow between red and white sectors two movement experiments were carried out. In the first, 12 vials containing *w* larvae were placed in the white light half of an experimental cage, and the same number of vials containing + larvae placed in the red light half. Flies emerged from the vials over the following 7 days and assorted themselves according to their habitat preference. The frequency of each allele among the offspring of the experimental flies was assessed by placing four sampling vials in each sector 1 week later. The frequency of *w* in the flies emerging from the sampling vials was 0.47 in the red light sector and 0.38 in the white light ($\chi^2 = 7.1$; $P < 0.01$). In the second experiment the 12 *w* vials were placed in the red light sector and the 12 + vials in the white light. Their offspring were sampled in the same way as in the first experiment; the frequency of *w* was 0.42 in the red light sector and 0.17 in the white light ($\chi^2 = 31.1$; $P < 0.001$). There is, therefore, the opportunity for substantial habitat selection by each generation between the environmental patches in the experimental cages. This is true whether or not the flies emerge into their preferred microenvironment, and must play a part in maintaining the differences in gene frequency between these patches shown in Fig. 2.

Associations between the extent of genetic polymorphism in natural populations and the ecological diversity of the habitats in which they live have often been reported. Populations of *Drosophila willistoni* living in florally complex environments have more inversion polymorphism than do those from simpler habitats¹¹ and there may be in some groups of animals a general association between niche breadth and morphological variation¹². Similarly, *Drosophila* populations exposed to diverse environments in the laboratory retain chromosomal and molecular polymorphism to a greater extent than do those kept in stable conditions^{13,14}. Natural and laboratory environments containing a variety of microhabitats offer the opportunity for genotypes to select those which are optimal. Differences in the tendency of individual members of natural populations to select particular microenvironments have indeed been found in *Drosophila*^{15,16}, and selection of the appropriate niche increases the relative fitness of, for example, melanistic moths, cryptically coloured molluscs and lizards, and perhaps also of enzyme polymorphisms in *Asellus* and *Drosophila* exposed to a variety of foods¹⁷⁻²¹.

Habitat selection can increase the fitness of an allele which is already advantageous. The experiments described here show

that it can also lead to the maintenance of polymorphism for an allele which is otherwise deleterious to its carriers.

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Vesicle formation and acetylene reduction activity in *Frankia* sp. CPI1 cultured in defined nutrient media

John D. Tjepkema, William Ormerod & John G. Torrey

Cabot Foundation for Botanical Research, Harvard University, Peter-sham, Massachusetts 01366

It has been reported that certain species of free-living *Rhizobium* were capable of producing nitrogenase¹⁻⁵, thereby demonstrating conclusively that the *nif* genes reside in the bacterial component of the dinitrogen-fixing symbiosis between *Rhizobium* and leguminous host plants. In the comparable dinitrogen-fixing symbiosis between the actinomycete *Frankia* and the actinorhizal woody dicotyledons that serve as nodulated host plants, the endophytic filamentous bacteria have only recently been brought into culture⁶⁻⁸. We report here evidence that free-living *Frankia* sp. grown in appropriate nutrient conditions show nitrogenase activity (acetylene reduction) associated with a distinctive morphological structure formed in effective nodules and also developed in culture.

Frankia sp. CpI1 isolated from root nodules of *Comptonia peregrina* and grown in liquid yeast-extract nutrient medium⁶ develops as a finely branching filamentous mat that produces enlarged intrahyphal or terminal sporangia of considerable size (up to 60 μ m) and complexity containing numerous 1-2- μ m diameter spores⁷. During the development of defined synthetic media for the culture of CpI1, it was observed that growth rates and morphological structures of the filamentous bacterium were affected by preparation procedures and chemical components of the medium. A defined medium was developed that elicited sporangium formation and also extensive formation of small spherical to ovoid structures borne terminally on short side branches, which we term vesicles, in agreement with earlier literature. Similar structures have been observed by others^{10,11}. Under Nomarski interference phase optics, the vesicles show thickened walls (Figs 1, 2), in contrast to immature developing sporangia. Thus far, they have proved difficult to preserve and section for transmission electron microscopy.

In the yeast-extract medium⁸, used for routine subculture, vesicles are not usually observed. Such cultures show no

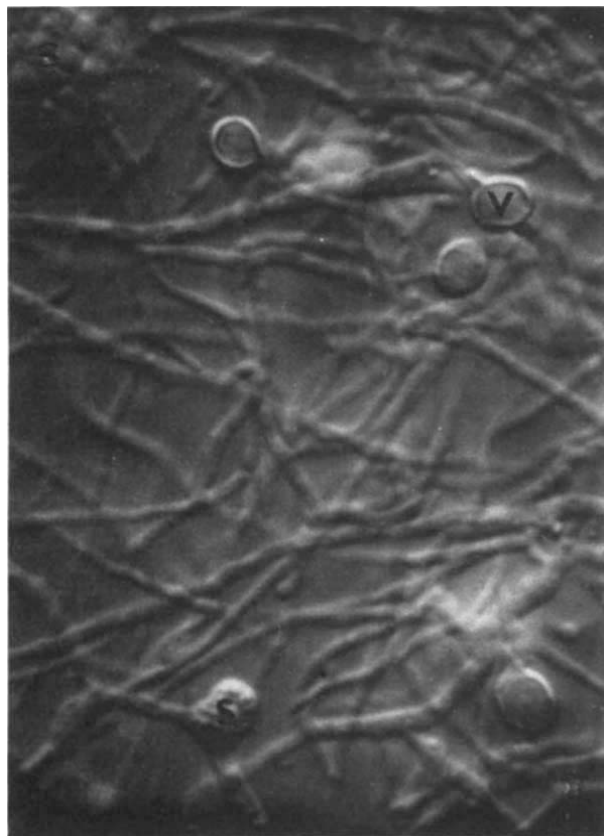


Fig. 1 A 4-week-old culture of *Frankia* sp. Cp11 grown in defined medium photographed with Nomarski phase interference optics. Branched bacterial filaments, enlarged sporangia (S) and a number of thick-walled vesicles (V) are visible. $\times 2,890$.

acetylene reduction activity. The following procedure was used to elicit vesicle formation. The filamentous mat from yeast-extract medium was collected after 4–6 weeks of standing culture in flasks. The mat was homogenized in a Potter–Elvehjem homogenizer and then washed three times with mineral salt solution from the defined medium (see below) and its packed cell volume measured. A sample of known volume was resuspended in mineral salt solution and added to test tubes containing 5 ml of defined medium with the following composition. Mineral salt solution (gm l^{-1}): 1.0 KH_2PO_4 , 0.1 $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 0.01 $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$, 0.01 FeNaEDTA ; 1 ml l^{-1} of the following micronutrient stock solution (gm l^{-1}): 2.86 H_3BO_3 , 1.81 $\text{Mn Cl}_2 \cdot 2\text{H}_2\text{O}$, 0.22 $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$, 0.08 $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$, 0.025 $\text{Na}_2\text{MoO}_4 \cdot 2\text{H}_2\text{O}$; 1 ml l^{-1} of the following stock solution (mg per 100 ml): 10 thiamine HCl, 50 nicotinic acid, 50 pyridoxine HCl; and 1.2 gm l^{-1} succinic acid, pH adjusted to 6.8. Note that the only nitrogen-containing compounds in this medium are EDTA and the vitamins, and that they contain only very small quantities.

Table 1 Time course of nitrogenase activity by *Frankia* sp. Cp11 cultured in defined medium

Time (days)	No. of vesicles per tube ($\times 10^6$)	Acetylene reduction (nmol per tube per day)
0	0	0
5	1.67 ± 0.35	7.3 ± 0.4
7	1.85 ± 0.26	11.9 ± 3.0
14	2.73 ± 0.41	2.9 ± 0.7
Defined medium plus 100 mg l^{-1} glutamic acid		
7	1.17 ± 0.41	6.6 ± 0.7

Values are $\pm$ s.e.m., $n = 5$.

In a typical experiment 0.8 ml packed cell volume of *Frankia* was used to inoculate 100 tubes (an inoculum of 8 μl packed cell volume per tube). Tubes were stoppered with cotton plugs and incubated at 25°C in the dark without agitation. For each sample used in time-course studies, five tubes were sampled for nitrogenase activity, using the acetylene reduction assay¹² with gentle agitation of the tubes. Vesicle counts were made by sonicating the contents of each tube for 30 s at 60 W and then counting in a Petroff-Hausser chamber under phase illumination.

Representative data are given in Table 1 showing the relationship between vesicle formation and acetylene-reducing activity over a culture period of 2 weeks. Cultures at the outset lacked vesicles and showed no acetylene reduction. Vesicle formation was first observed at 5 days and the number of vesicles per tube increased with time. Acetylene reduction occurred only in cultures with vesicles and this activity increased roughly in proportion with the number of vesicles formed between days 5 and 7. Nitrogenase activity decreased at 14 days, even though vesicle numbers continued to increase. The cause of this decrease is not known but may have been due to accumulation of inhibitory substances. The maximum activity recorded in any of these experiments was 44 nmol per tube per day. One experiment was conducted with *Frankia* sp. Ar13 from *Alnus rubra*⁷ and this also resulted in vesicle formation and nitrogenase activity.

The nitrogenase activity observed is consistent with that of intact nodules, which is $\sim 10 \mu\text{mol C}_2\text{H}_4 \text{ h}^{-1} \text{ cm}^{-3}$ of nodule volume. We estimate that vesicles make up about 1% of nodule volume; assuming that the vesicles are the site of nitrogenase, their activity would be 1,000 $\mu\text{mol C}_2\text{H}_4 \text{ h}^{-1} \text{ cm}^{-3}$ of vesicle volume. Using a vesicle diameter of 2.5 μm , the highest activity we have observed (Table 2) is equivalent to 180 $\mu\text{mol h}^{-1} \text{ cm}^{-3}$, or 18% of the value estimated for vesicles in nodules. For comparison, we calculate that bacteroids in legume nodules have a nitrogenase activity of $\sim 100 \mu\text{mol C}_2\text{H}_4 \text{ h}^{-1} \text{ cm}^{-3}$ of bacteroid volume.

It is very unlikely that the nitrogenase activity of our cultures was due to contaminating organisms as activity was observed only in cultures with vesicles and was correlated with the number of vesicles. Further evidence is the lack of growth when Cp11 is inoculated into Difco nutrient broth, absence of turbidity in all cultures, and lack of growth and nitrogenase activity in anaerobic conditions.

Inoculum density was an important variable not easily controlled because of the difficulty in accurately measuring



Fig. 2 A young culture of Cp11 showing a bacterial filament with an immature developing sporangium (S) side by side with a mature thick-walled vesicle (V). Nomarski optics. $\times 4,375$.

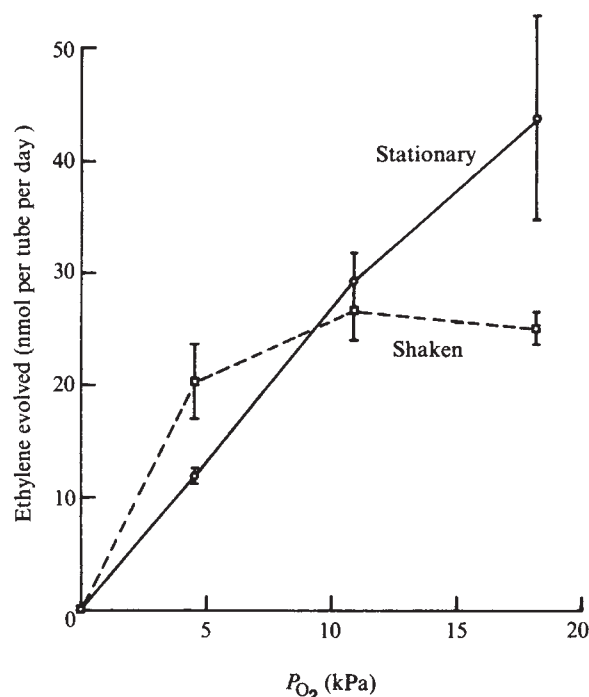


Fig. 3 Acetylene reduction by cultures of *Frankia* as a function of P_{O_2} . One set of tubes was shaken throughout the experiment and the other was stationary. From 0 to 19 days after inoculation, the tubes were incubated in air. They were then gassed with mixtures of air, N_2 , and 10% C_2H_2 . Vertical bars represent $\pm$ s.e.m.; $n = 5$.

actinomycete mass and growth. Positive correlations were found between inoculum density, vesicle number, and acetylene reduction activity (Table 2). Experimental differences in similar tests were probably most often attributable to variation in initial inoculum density.

In several series of experiments we tested the effect of additions of inorganic or organic components to the defined medium on acetylene reduction activity. Cellobiose added at 1% (w/v) completely inhibited vesicle formation and acetylene reduction over a period of 3 weeks. Glutamic acid added at 100 mg l^{-1} reduced the number of vesicles and nitrogenase activity by about half (Table 1). Ammonium chloride (54 mg l^{-1}) completely suppressed vesicle formation and acetylene reducing activity. From the experiments performed to date, medium additions which inhibit acetylene reduction activity do so by suppressing vesicle formation. We have no information to suggest that the ammonium chloride and glutamate inhibited nitrogenase activity *per se*.

Finally, in view of the known instability of the nitrogenase enzyme to molecular oxygen¹³, we were interested in studying the effects of P_{O_2} on acetylene reduction activity in cultures of Cp11 in defined medium. As shown in Fig. 3, acetylene reduction by shaken cultures was little affected by the P_{O_2} values examined, while stationary cultures were oxygen limited. Apparently, the enzyme within the vesicle is protected from

oxygen in some way, either by special metabolism within the vesicle or by the relative impermeability of the thickened wall. This suggests analogy with heterocyst formation and function in the blue-green algae.

These experiments establish several new and interesting facts. They give the first published evidence that free living *Frankia* sp. are capable of nitrogenase activity in appropriate nutrient conditions. The positive correlation between vesicle formation and acetylene reduction activity *in vitro* gives strong support to the already expressed view^{8,13-17} that vesicles are the site of nitrogenase activity in actinorhizal plants. Our experiments further show that vesicle formation is elicited or made possible by the physico-chemical conditions which surround the micro-organism *in vitro*, a fact which raises interesting questions about the lack of vesicle formation in certain incompatible cross inoculations^{16,18}. These observations open up a new approach to the problems of structure and function in these important symbiotic nitrogen fixing relationships.

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Complete reversal of antero-posterior polarity in a centrifuged insect embryo

Klaus-Gerhard Rau & Klaus Kalthoff

Department of Zoology, University of Texas at Austin, Austin, Texas 78712

Spatial pattern formation during embryogenesis is ascribed to differential gene expression, which in turn is thought to result in part from interactions of nuclei with cytoplasmic determinants¹. In the chironomid midge *Smittia*, and probably in other dipterans as well, blastoderm cells seem to make an early decision as to whether they contribute to cephalic and thoracic or to abdominal (and possibly thoracic) structures². Inactivation or translocation of cytoplasmic components involved in this antero-posterior decision could conceivably lead to duplications of head and thorax, or abdomen, or to complete but inverted embryos forming the head posteriorly and the abdomen anteriorly in the egg. Whereas the former two malformations have been described^{3,4}, completely inverted embryos are reported for the first time, to our knowledge, in this letter. Reversal of partial germ bands has previously been observed following combined ligation and cytoplasmic translocation in eggs of the leaf hopper, *Euscelis plebejus*⁵.

Table 2 Effect of inoculum density on nitrogenase activity by *Frankia* sp. Cp11 cultured in defined medium

Time (days)	Inoculum density (%)	No. of vesicles per tube ($\times 10^6$)	Acetylene reduction (nmol per tube per day)
0	100	0	0
7	100	—	1.4 ± 0.3
14	100	0.95 ± 0.09	25.0 ± 3.1
14	50	0.56 ± 0.04	20.4 ± 3.9
14	25	0.23 ± 0.04	9.1 ± 2.7
14	10	0.05 ± 0.01	1.0 ± 0.7

Values are $\pm$ s.e.m., $n = 5$.

Smittia eggs were centrifuged during early intravitelline cleavage (stage P₂, two pole cells) for 20 min at 6,700g and 20 °C. During centrifugation, eggs were lined up, with their posterior poles pointing in the direction of the centrifugal force, in a tightly fitting groove between microscope cover slips⁴. Eggs recovered from the centrifuge were screened for proper stratification as shown in Fig. 1*i*; obliquely stratified eggs were removed. Out of 6,206 centrifuged eggs, 3,140 survived and formed analysable germ bands. Among these, we found 1,713 normal embryos (Fig. 1*d, f*), 1,341 double cephalons (Fig. 1*a*), 55 double abdomens (Fig. 1*b*) and 31 inverted embryos (Fig. 1*c, e*). As controls, 581 embryos were centrifuged with their anterior poles pointing in the direction of the centrifugal force. All of the 333 survivors developed into normal embryos. In similar experiments using higher centrifugal forces, centrifugation with the anterior pole pointing in the direction of the centrifugal force resulted in low yields of double cephalons and double abdomens⁴.

Inverted embryos were identified using the following criteria: (1) the contour of the chorion (egg shell) is more pointed at the posterior and more blunted at the anterior pole (Fig. 1*a-f* and *i*); (2) the micropyle at the anterior pole is visible as a small but distinct dark area in transmitted light under the compound microscope (Fig. 1). With Nomarski optics, the micropyle region shows a bright blue coloration, depending on the position of the second Wollaston prism, and the angle between the polarisator plane and the plane tangential to the anterior pole of the embryo. Variation of this angle leads to appearance and disappearance of the blue colour in the micropyle every 90°; and (3) the stratification of yolk components in centrifuged embryos is maintained until after germ anlage formation; the gray and opaque proteid spheres accumulated centrifugally are easily distinguished from the lipid droplets in the centripetal region which have a brownish hue. In embryos of normal orientation, the head lobes formed in the lipid-containing region, while the 'Schwanzwulst' (posterior end of germ anlage) originated in the proteid-containing region (Fig. 1*f*). Conversely, the inverted embryos developed with their head lobes in the proteid and the Schwanzwulst in the lipid region (Fig. 1*e*). Application of criteria 1-3 led in no case to contradictory conclusions.

It is important to realize that the polarity inversions did not result from turning of the entire embryos inside their egg shells. This possibility is excluded by criterion (3) which indicates the orientation of the embryo relative to the yolk-rich endoplasm. The development of inverted embryos was also observed, in eleven cases, from the very beginning of germ anlage formation. The ensuing formation of amnion and serosa, embryonic rotations, germ band extension and contraction, dorsal closure, and spiralization of inverted embryos appeared normal or nearly so. More than half of the inverted embryos hatched and showed normal larval movements.

A hypothesis to explain the formation of four different segment patterns (normal embryos, double cephalons, double abdomens, and inverted embryos) after centrifugation is shown diagrammatically in Fig. 2. The hypothesis builds on a model developed earlier⁶, according to which the potential for abdomen formation (p, p') is present not only in the posterior but also

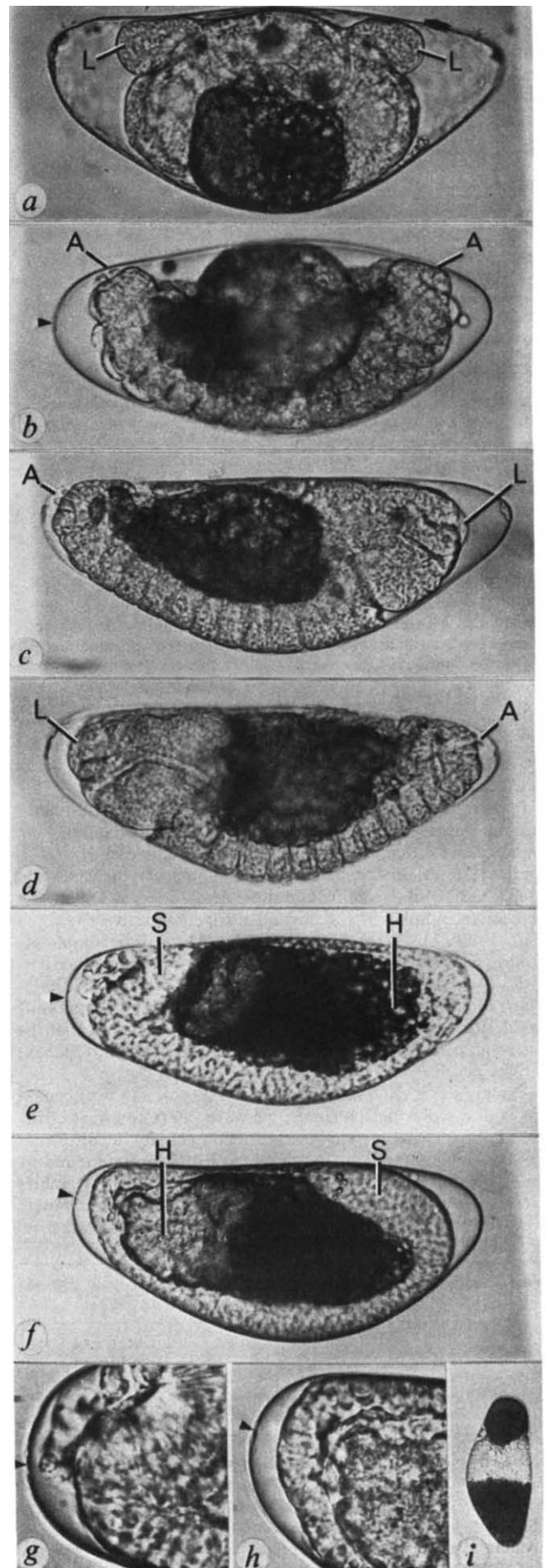


Fig. 1 Normal and abnormal segment patterns of *Smittia* embryos developing from centrifuged eggs. *a-h*, Ventral side down, anterior pole of egg shell to the left. *a-d*, Embryos after shortening of germ band, 53-78 h after deposition: *a*, double cephalon; *b*, double abdomen; *c*, inverted embryo; *d*, normal embryo. *e-h*, Embryos during formation of germ anlage, 18-19 h after deposition: *e, g*, inverted embryo; *f, h*, normal embryo; *i*, centrifuged egg, posterior pole (down) was facing in direction of centrifugal force. Note centrifugal accumulation of proteid spheres (at bottom in *i*, to right in *e* and *f*) and centripetal accumulation of lipid droplets. L, Labrum (anteriormost head structure); A, anal papillae marking abdominal end; S, Schwanzwulst (posterior bulge of germ anlage); H, head lobes, in lateral position, displacing yolk material in between; $\blacktriangle$, micropyle at anterior end of egg shell.

in the anterior egg half. This is shown by the ability of isolated anterior fragments to form abdomens⁷, and is also reflected in the synchronous and symmetrical development of double abdomens. The formation of head and thorax is ascribed to the superimposed activity of 'anterior determinants' which have been characterized as cytoplasmic ribonucleoprotein particles⁶. This is mainly based on the formation of double abdomens after application of RNase or UV light to the anterior pole region, on the action spectrum for UV-ray induction of double abdomens, and on the subcellular localization of the effective targets of UV light. Although the exact function of the anterior determinants remains to be elucidated, it is assumed that their activity in crucial regions of an embryonic half has to surpass a threshold level for head and thorax to be formed there. Sub-threshold levels of activity are thought to result in abdomen formation.

Centrifugation of *Smittia* eggs obviously causes stratification of yolk components (Fig. 1i) and probably of nuclei, and hence displacement of other egg components. In addition, disruption of some cytoskeletal structures and other less obvious consequences may occur. Redistribution of egg components after centrifugation involves disappearance of the intermediate layer of yolk-free cytoplasm within 4 h, and a somewhat abnormal blastoderm formation which seems to proceed from the egg equator to the poles⁴. These processes may lead, in the embryo, to various spatial distributions of anterior determinants whose activity may also depend on their intracellular environment and/or the time of their interaction with nuclei. As an overall result, one of the following four situations could arise with respect to anterior determinant activity. Above-threshold activity in both anterior and posterior halves (Fig. 2c), below-threshold activity in both anterior and posterior halves (Fig. 2d), above-threshold activity in the posterior half only (Fig. 2e), or above-threshold activity in the anterior half only (Fig. 2f). These situations, according to our model, should lead to formation of double cephalons, double abdomens, inverted embryos, or

normal embryos, respectively. In fact, all of these segment patterns have been observed after centrifugation.

Moreover, this hypothetical explanation of our centrifugation experiments allows us to make four predictions as to the modifying effects of UV light (thought to inactivate anterior determinants) before or after centrifugation. (1) UV-ray irradiation of the anterior pole region before centrifugation should cause a major increase in the yield of double abdomens, and the disappearance of normal embryos, double cephalons, and inverted embryos; (2) UV-ray irradiation of the posterior pole before centrifugation should have little or no effect; (3) UV-ray irradiation of the anterior pole region after redistribution should reduce the yields of normal embryos and double cephalons, and enhance the yields of double abdomens and inverted embryos; and (4) UV-ray irradiation of the posterior pole region after redistribution should enhance the yields of normal embryos and double abdomens at the expense of double cephalons and inverted embryos. These predictions will be tested.

Incidentally, we expected and first discovered inverted embryos after centrifugation and subsequent UV-ray irradiation of the anterior pole region. In these conditions, they occurred more frequently than after centrifugation alone. Possibly they were also produced in earlier centrifugation experiments⁴ but overlooked because they were not anticipated. *Note added in proof:* We have learned that inverted embryos have been generated earlier by centrifugation of *Chironomus samoensis* embryos⁸.

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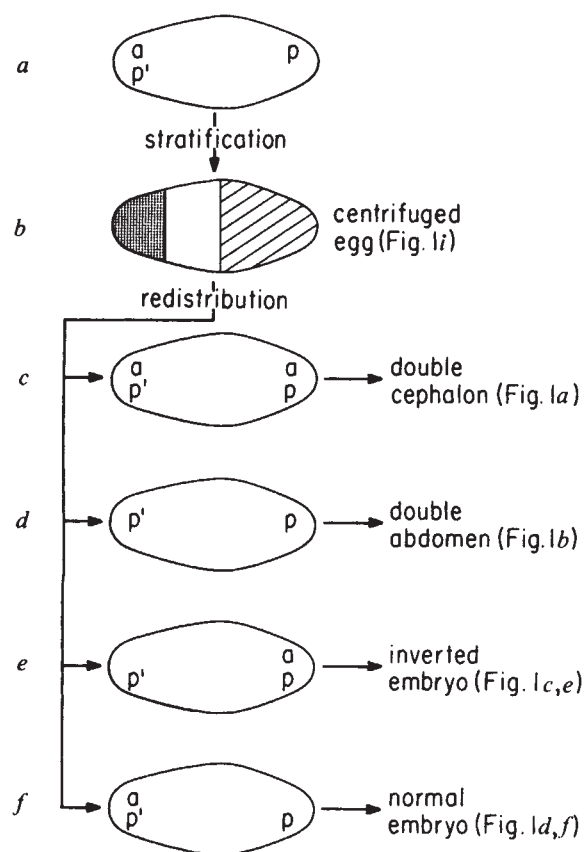


Fig. 2 Hypothesis explaining the formation of different segment patterns by different distributions of anterior determinant (a) activity after stratification and redistribution of egg material. p, p', Posterior factors.

Cytochalasin D does not produce net depolymerization of actin filaments in HEP-2 cells

Allen Morris* & Janet Tannenbaum†‡

* Department of Biology, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139

† Department of Pathology, College of Physicians and Surgeons, Columbia University, New York, New York 10032

The altered morphology, disappearance or 'disruption' of actin filaments (microfilaments) in cells treated with cytochalasin¹ has sometimes been attributed to depolymerization of filamentous actin (F-actin) to its globular subunit (G-actin), but attempts to confirm that mechanism have been inconclusive. Treatment of purified actin filaments with cytochalasin B (CB) decreased their viscosity^{2,3}, consistent with depolymerization, which was not, however, revealed by electron microscopy⁴, although the filaments appeared abnormal⁵. CB also increased the ATP-ase activity of F-actin, suggesting that it had been destabilized³, while actin filaments in the acrosomal process were not depolymerized⁶. CB or cytochalasin D (CD) can dissolve actin gels (reviewed in ref. 7, see also refs 8 and 9) without depolymerizing their filaments. The 'disrupted' actin structures in CD-treated cells bound heavy meromyosin¹⁰, indicating that at least some of the cellular actin was filamentous. Using a rapid assay for G- and F-actin in cell extracts, based on the inhibition of DNase I¹¹, we have found that neither short- nor long-term exposure of HEP-2 cells to CD produce net depolymerization of actin filaments.

‡ To whom correspondence should be addressed.

The human tumour cell line HEP-2 (ATCC CCL23) was grown in monolayers in minimal essential medium (Earle's salts) supplemented with 10% newborn calf serum, penicillin (100 U ml^{-1}), streptomycin ($100 \text{ g } \mu\text{l}^{-1}$) and Fungizone ($0.25 \text{ } \mu\text{g ml}^{-1}$, Gibco). Cells were planted at a density of 5×10^5 per 60 mm plastic Petri dish (Falcon), incubated in a humidified atmosphere of 5% carbon dioxide–95% air at 37°C , and used within 2–3 days (sub-confluent). CD (Sigma) was stored as a 1 mg ml^{-1} stock solution in DMSO and diluted into Earle's balanced salt solution (EBSS, Gibco) to prepare a working stock solution immediately before use. Treatment with CD was initiated by refeeding cells with fresh medium containing CD (or an equivalent amount of DMSO for controls). After incubation as indicated in Table 1, the monolayers were drained, rinsed quickly with warm EBSS and scraped into 0.3–0.4 ml of 5 mM potassium phosphate, pH 7.6, 150 mM NaCl, 2 mM MgCl_2 , 0.2 mM dithiothreitol, 0.2 mM ATP, 0.5% Triton X-100 and 1 mM PMSF (added immediately before use). Samples of 10–20 μl of this cell lysate were used for the DNase I assay as described by Blikstad *et al.*¹¹. Samples of lysate mixed with the guanidine hydrochloride depolymerizing solution¹¹ were sheared in a syringe with a 26-gauge needle to decrease viscosity. All other procedures were as described by Blikstad *et al.*¹¹. Chemicals were reagent grade; highly polymerized calf thymus DNA (Sigma) and bovine pancreas DNase I (Sigma DN-Cl, lyophilized powder) were used. Protein concentration was estimated by the method of Bradford¹², using the Bio-Rad Protein Assay reagent and bovine gamma globulin or serum albumin standards.

HEP-2 cells treated with CD exhibited characteristic morphological effects, the protrusion of zeiotic knobs from the cell surface^{13,14} at all three concentrations of CD tested. As Table 1 shows, short-(3.75 h) or long-term (24–27 h) treatment with CD did not result in a net depolymerization of actin. Although there was some variability in the results, the long-term effect of CD appeared to be a net decrease in the proportion of

Table 1 Effect of treatment with CD on proportion of G-actin and total actin in HEP-2 monolayers

Expt no.	Treatment	% G-actin	Units actin $\mu\text{g cell protein}^{-1}$
1	DMSO, 0.1% (3.75 h)	67.8 ± 16.4	ND
	CD, $0.5 \text{ } \mu\text{g ml}^{-1}$ (3.75 h)	62.5 ± 5.8	ND
	CD, $1.0 \text{ } \mu\text{g ml}^{-1}$ (3.75 h)	69.3 ± 13.5	ND
2	DMSO, 0.05% (24.0 h)	57.5 ± 5.4	ND
	CD, $0.5 \text{ } \mu\text{g ml}^{-1}$ (24.0 h)	56.4 ± 7.4	ND
3	DMSO, 0.1% (26.75 h)	61.4 ± 4.4	ND
	CD, $0.5 \text{ } \mu\text{g ml}^{-1}$ (26.75 h)	42.3 ± 9.0	ND
	CD, $1.0 \text{ } \mu\text{g ml}^{-1}$ (26.75 h)	36.2 ± 4.7	ND
4	DMSO, 0.1% (27.0 h)	63.5 ± 5.0	2.2 ± 0.2
	CD, $0.2 \text{ } \mu\text{g ml}^{-1}$ (27.0 h)	47.3 ± 6.7	2.6 ± 0.1
	CD, $0.5 \text{ } \mu\text{g ml}^{-1}$ (27.0 h)	52.4 ± 6.3	2.7 ± 0.3
	CD, $1.0 \text{ } \mu\text{g ml}^{-1}$ (27.0 h)	49.4 ± 5.3	2.8 ± 0.2

Replicate monolayers of HEP-2 cells were incubated with CD or DMSO solvent in medium as indicated (in experiments 1, 3, 4, all concentrations of CD were prepared in 0.1% DMSO). Cells were then collected and lysed as described in the text. Samples of lysate were assayed for their DNase I inhibitor activity before (a measure of G-actin) and after (a measure of total actin) addition of the guanidine hydrochloride depolymerizing buffer. For each determination, the amount of sample used was adjusted to produce inhibition of the DNase I within the linear range of the assay (30–70% inhibition). The percentage of G-actin was calculated by comparing the G-actin inhibitory activity with the total actin inhibitory activity for equal amounts of cell material. Data shown are each averages of three or four determinations per sample, $\pm$ s.d. Each experiment was performed with a separate set of replicate monolayers. In experiment 4, protein concentrations in the lysates were, in the order shown: 2.35, 2.25, 2.15 and 2.10 mg ml^{-1} . Values for units of actin per μg protein were calculated from the total actin in each sample (after guanidine hydrochloride depolymerization) for 10- μl volumes; 1 U inhibits 1% of the $1 \text{ } \mu\text{g}$ of DNase in the standard assay. ND, not determined.

Table 2 Effect of continued exposure to CD during collection and lysis of CD-treated HEP-2 cells

Expt no.	Treatment	% G-actin	Units actin $\mu\text{g cell protein}^{-1}$
1	DMSO, <i>in vivo</i> and <i>in vitro</i>	80.0 ± 8.8	3.3 ± 0.2
	CD, <i>in vivo</i> (DMSO <i>in vitro</i>)	68.1 ± 10.0	4.1 ± 0.3
	CD, <i>in vivo</i> and <i>in vitro</i>	66.8 ± 4.2	3.9 ± 0.1
2	DMSO, <i>in vivo</i> and <i>in vitro</i>	86.0 ± 12.0	3.8 ± 0.3
	CD, <i>in vivo</i> (DMSO <i>in vitro</i>)	69.9 ± 6.3	4.6 ± 0.2
	CD, <i>in vivo</i> and <i>in vitro</i>	69.4 ± 10.8	4.5 ± 0.3

Replicate monolayers of HEP-2 cells were incubated with 0.1% DMSO or CD ($1.0 \text{ } \mu\text{g ml}^{-1}$) in 0.1% DMSO for 17.0 h (experiment 1) or 19.5 h (experiment 2) (*in vivo* treatment). Cells were then rinsed and collected as indicated in the text, except that rinsing and lysing solutions contained 0.1% DMSO (cells exposed to DMSO '*in vitro*') or $1.0 \text{ } \mu\text{g ml}^{-1}$ CD in 0.1% DMSO (cells exposed to CD '*in vitro*'). Thus, for cells treated with CD *in vivo* and *in vitro*, exposure to CD was continuous to prevent possible recovery from effects of the drug. All other details are given in the legend for Table 1. Separate stocks of HEP-2 cells were used in the experiments for Tables 1 and 2; possibly differences between these two stocks account for the higher % G-actin in Table 2 compared with Table 1. The values for units of actin per μg protein are not directly comparable between Tables 1 and 2, because different lots of DNase I and DNA substrate were used for each.

G-actin to total actin in the cell. Calculation of the total units of actin per μg of protein (Tables 1, 2) suggested that long-term treatment with CD resulted in an increased accumulation of actin in HEP-2 cells; this effect has been observed repeatedly in further experiments (unpublished data of J.T. and G. C. Godman). Addition of $1 \text{ } \mu\text{g ml}^{-1}$ of CD (DMSO for controls) to the EBSS and the Triton-containing buffer used during collection and lysis of CD-treated cells (to prevent any possible reversal of the effects of CD during these steps) did not alter these results (Table 2).

The approximate concentration of G-actin in the lysates can be determined from Table 1 and our estimate that 6.8% of the protein in DMSO-treated HEP-2 cells is actin (unpublished observations based on ^{35}S -methionine labelling of HEP-2 cells and isolation of actin by polyacrylamide gel electrophoresis). For the protein concentrations in experiment 4, we calculated that the lysates contained G-actin at a concentration of $102 \text{ } \mu\text{g ml}^{-1}$ (DMSO control) and at 87, 94 and $90 \text{ } \mu\text{g ml}^{-1}$ (respectively, for CD at 0.2, 0.5 and $1 \text{ } \mu\text{g ml}^{-1}$; all values were corrected for the increase in total actin per μg protein compared with DMSO controls). All these values are greater than the critical concentration reported for various non-muscle actins in similar conditions¹⁵, possibly because the cellular G-actin is associated with inhibitors of polymerization such as profilin¹⁶.

These results indicate that CD does not cause a massive net depolymerization of actin filaments *in vivo*. It should be noted, however, that the DNase I assay used does not distinguish the length of the actin filaments and therefore cannot test a proposed mechanism involving the scission of F-actin to shorter lengths of filament¹⁷. In addition, the data do not exclude the possibility that CD converts actin microfilaments to some other configuration rather than depolymerizing them.

Cytochalasins B, E and D have been reported to inhibit the *in vitro* induction of F-actin polymerization by a high molecular weight complex which binds dihydro-CB at high affinity¹⁸. Similarly, CD inhibited polymerization of actin nucleated by F-actin^{19,20}. On the basis of these observations, it was suggested that cytochalasins might act by inhibiting polymerization of actin microfilaments in the cell. Because the actin in non-muscle cells probably undergoes cycles of polymerization and depolymerization (reviewed in ref. 21), this proposal implies that cytochalasin should eventually produce a net increase in the proportion of G-actin in the cell by inhibiting its repolymerization during the normal cycle. Our failure to detect such an increase, even after 27 h of treatment with CD at concentrations sufficient to alter cell morphology, tends to contradict this

hypothesis. However, because the DNase I assay only determines the net balance of F and G-actin at the time the cells are collected, our experiments do not test the possibility that CD inhibited the nuclei-induced, rapid polymerization of actin in the cell but also allowed (and perhaps accelerated^{22,23}) a compensating polymerization of this actin on to the more slowly growing end of actin filaments.

Our observation that CD does not cause a net depolymerization of actin filaments *in vivo* argues against any mechanism for the action of cytochalasin on cells which is based on the replacement of microfilaments by depolymerized actin. In particular, it suggests that normal levels of actin microfilaments would be available for participation in an

energy-dependent contractile response of the cells to treatment with CD, as previously proposed^{10,13,14,24,25}.

In summary, treatment of live HEP-2 cells with CD did not cause a net depolymerization of filamentous actin. Rather, there seemed to be a small increment in the proportion of filamentous actin in cells treated for 27 h and an increase in the total content of actin. The possibility that an accumulation of F-actin in CD-treated cells corresponds to the actin-containing masses (reviewed in ref. 25) seen in such cells is being investigated.

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Lack of heterogeneity in anti-hapten antibodies of a phylogenetically primitive shark

O. Mäkelä

Department of Bacteriology and Immunology, University of Helsinki, SF-00290 Helsinki 29, Finland

G. W. Litman

Sloan-Kettering Institute for Cancer Research, Walker Laboratory, Rye, New York 10580

Individual mammals have the capacity to express at least one million distinct antigen binding specificities, implying a high degree of structural heterogeneity in the variable heavy and light chain (V_H and V_L) portions of the antibody molecules. Studies of higher vertebrate species suggest that this heterogeneity is created both through a sizeable repertoire of germ-line V_H and V_L genes¹ and through random rearrangements of V and joining genes^{2–4}. Additional somatic mechanisms probably also contribute to the ultimate heterogeneity; one-third of murine plasmacytomas producing λ_1 immunoglobulin carry a somatically mutated Ig1-V gene^{5,6}. The relative contributions of these various mechanisms to the overall immunoglobulin variability are difficult to evaluate. The production of different antibodies to a defined determinant in different individuals of an inbred mouse strain [for example, (3-iodo-4-hydroxy-5-nitrophenyl) acetyl (NIP) in CBA mice⁷] suggests the involvement of somatic mutations or rearrangement but does not rule out the possibility that each individual CBA mouse expresses only a small random fraction from a large germ-line repertoire of V genes determining different anti-NIP binding sites. The opposite finding, that different individuals produce nearly identical antibodies to a defined determinant, would suggest the presence and expression of a limited number of germ-line genes without somatic alterations. Data presented here suggest that primitive sharks (*Heterodontus francisci*)⁸ produce such antibodies to the hapten furyloxazalone.

Heterodontus (0.2–0.5 kg) of estimated age 2–3 yr were maintained as described previously⁹. Intramuscular immuniza-

tion with *Brucella abortus* to which 2-furyloxazalone (furyl Ox) was coupled¹⁰ was carried out on day 0 and day 30. Inbred male rats of the SDK strain (0.34–0.43 kg) aged 3–4 months were immunized with the same dose and timing. Antibody to furyl Ox was measured by neutralization of bacteriophage T4 coupled with furyl Ox-aminocaproyl (furyl Ox-cap-T4)¹⁰. The shark antibody was found to reside in the high molecular weight serum fraction as judged by sensitivity to 0.1 M 2-mercaptoethanol and the 19S sedimentation constant of the phage neutralizing activity. Antibodies of rat sera were found in both high and low molecular weight fractions. However, in the experiments reported we have used the 19S fraction prepared by sucrose density gradient centrifugation, or for the day 9 sera, antibody remaining after elimination of IgG by incubation of 1.0 ml of 20% serum with 0.7 ml of packed protein A-Sepharose for 1 h at 20 °C (ref. 11).

The shark antisera discriminated between the structurally different haptens furyl Ox and azobenzene arsonate (Ars) in a manner comparable to rat antisera (Table 1). The anti-furyl Ox titres after immunization with furyl Ox were 170 times higher than the anti-Ars titres. The only anti-Ars serum that we tested exhibited a 56-fold difference in the reverse direction.

The affinity and fine specificity of the antibodies were characterized by the inhibition of the phage neutralization using seven structurally related haptens. The concentration of hapten required to reduce the antibody titre by 50% was termed the IC_{50} ; a low IC_{50} value indicated a high affinity and vice versa¹².

Table 1 Anti-furyl Ox or anti-Ars titres of *Heterodontus* or rat antisera

Day	Anti-furyl Ox titres at various days post-immunization				Anti-Ars titres
	—	9	20	55	55
<i>Heterodontus</i>	1,300	—	18,000	143,000	270
Rat (whole sera)	—	4,600	12,000	386,000	160

The data are geometric mean titres of four *Heterodontus* and six rat sera. The antibodies were measured by neutralization of bacteriophage T4 coupled with furyl Ox-aminocaproyl¹⁰ or BOC-ABA-tyrosine¹⁶. An anti-Ars serum obtained on day 180 of an immunization course with *B. abortus* coupled with azobenzene arsonate⁹ was tested with the same phage conjugates; its anti-furyl Ox titre was 830 and anti-Ars titre 15,000.

This analysis revealed three differences between shark and rat 19S antibodies (Fig. 1).

The shark antibodies had lower affinities for the immunizing hapten feryl Ox-cap (log mean IC_{50} 35 μ M) than did rat antibodies (IC_{50} 0.82 μ M). It might be argued that the IC_{50} values cannot be used to compare affinities because the degree of polymerization may differ in the rat and the shark IgM. Even if this were true, shark antibodies are likely to be less polymeric than rat antibodies. (Rat antibodies are pentameric, some fishes have tetrameric antibodies¹³.) The true species difference of affinities would then be greater than the IC_{50} values indicate. The low affinity may be characteristic of all piscine antibodies¹³.

Another difference between the two species is in the discriminating power of the antibodies. Shark antibodies had rather similar affinities for the immunizing hapten and all six analogues. The average relative affinities for the analogues only varied from 37% (styryl Ox-cap) to 150% (fluorophenyl Ox-cap), whereas rat antibodies discriminated strongly between feryl Ox-cap and some of the analogues. For example, the average affinity for nitrophenyl Ox-cap and styryl Ox-cap was less than 1/10th of the average affinity for feryl Ox-cap.

The third characteristic of shark antibodies which distinguishes them from rat antibodies was a constant pattern of relative affinities for the structural analogues of feryl Ox-cap. This species-specific pattern was exhibited by all eight sera representing four individual fish and two time points post-immunization. For example, the affinity for fluorophenyl Ox-cap was always ~150% and the affinity for styryl Ox-cap was always ~37% relative to the affinity for feryl Ox-cap. The greater vertical variations of rat values than shark values shown in Fig. 1 indicate that the rats showed no such species-specific pattern. Even more striking differences could be seen when comparing individual rats: thus, antibodies of one rat had a three times higher affinity for iodophenyl Ox-cap than for feryl Ox-

cap whereas antibodies of another rat had a 40-fold difference in the reverse direction. A modest increase of affinities for feryl Ox-cap was observed in the rats from day 9 to day 55 but the differences were not statistically significant.

Thus, we found that ferylloxazolone induced a similar low-affinity antibody population of limited discriminating capacity in four individual sharks and that this population did not change detectably over a 25-day period of immunization. By contrast, rat IgM antibodies elicited in response to immunization with the same bacterium-hapten conjugate had a high affinity for the immunizing hapten but a low affinity for some of its chemical analogues; differences between individual animals were large. These data are consistent with the presence in *Heterodontus* of a stable, limited repertoire of antibody molecules of probable germ-line origin, and expand preliminary structural observations which support this hypothesis^{14,15}. *Heterodontus* may represent a valuable developmental model for examining the genetic processes which have been involved in the diversification of the variable-region gene pool during vertebrate evolution.

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Phenypressin (Phe²-Arg⁸-vasopressin), a new neurohypophyseal peptide found in marsupials

M. T. Chauvet, D. Hurpet, J. Chauvet & R. Acher

Laboratory of Biological Chemistry, University of Paris VI, 96 Boulevard Raspail, Paris 75006, France

Recent investigations on marsupial neurohypophyseal hormones have revealed that species belonging to the Australian family Macropodidae and the American family Didelphidae have, apart from an oxytocin-like hormone, two vasopressin-like peptides which can be separated either by ion-exchange chromatography or chromatoelectrophoresis^{1–3}. The major pressor hormone of two Australian species, the red kangaroo (*Macropus rufus*) and the tammar (*Macropus eugenii*), has been identified as lysine vasopressin by its amino acid sequence and its pharmacological properties^{1,2}. We report here that the minor pressor hormone, which chromatographs on Amberlite CG-50 like arginine vasopressin^{1,2}, differs from it in sequence only at position 2 where phenylalanine replaces the tyrosine of arginine vasopressin⁴.

The procedure described previously for isolation of lysine vasopressin^{1,2} was used for the purification of the minor pressor hormone. Acetone-desiccated posterior pituitary lobes (oxytocic activity 0.7 U mg⁻¹ and pressor activity 2.9 U mg⁻¹ for red kangaroo, oxytocic activity 0.8 U mg⁻¹ and pressor activity 2.3 U mg⁻¹ for tammar) were extracted by 0.1 M HCl (1 ml for

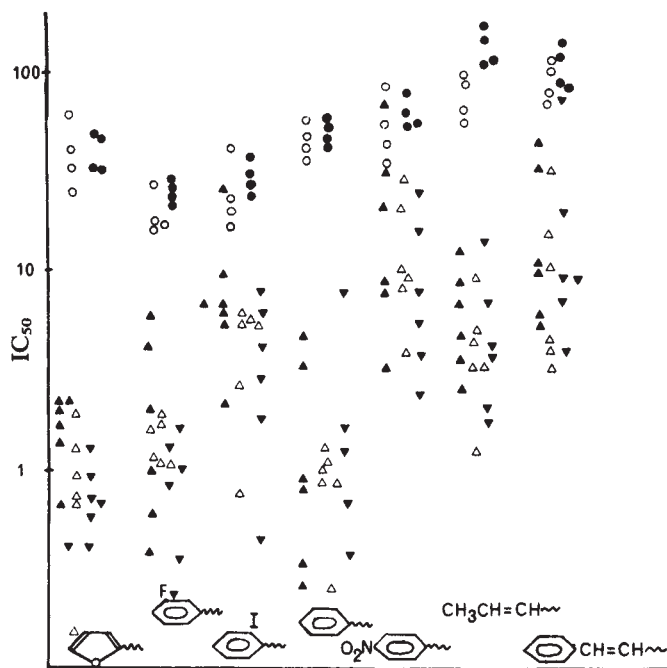


Fig. 1 Fine-specificity characteristics of *Heterodontus* and rat antibodies (19S). Relative affinities for seven different oxazolones were determined. Each symbol represents an IC_{50} value of an antiserum or its 19S fraction for the compound illustrated below the column. All compounds shared the oxazolone- ϵ -aminocaproyl (Ox-cap) structure. The structures illustrated were attached to the C-2 of Ox-cap. From the left the compounds are: feryl Ox-cap (closely resembles feryl Ox-lysine, the immunizing determinant), fluorophenyl Ox-cap, iodophenyl Ox-cap, phenyl Ox-cap, nitrophenyl Ox-cap, propyl Ox-cap and styryl Ox-cap. Circular symbols represent *Heterodontus* sera from day 20 (○) or day 55 (●). Triangles represent rat sera from day 9 (▲), day 20 (△) or day 55 (▼).

8 mg) for 4 h at 4 °C. Two experiments were carried out for each species with nine (42 mg) and 14 (67.7 mg) glands of red kangaroo and 24 (40.1 mg) and 33 (51 mg) glands of tammar. Supernatant solutions, separated by centrifugation, were subjected to molecular sieving on a column (1 × 120 cm) of Biogel P₄ equilibrated with 0.1 M acetic acid. Proteins were excluded and an oxytocic peak (yield in activity 78–83%) was separated from a pressor peak (yield in activity 77–87%). Fractions of each peak were pooled and solutions concentrated to a small volume by lyophilization. Materials were subjected to paper chromatoelectrophoresis in conditions described previously⁵. Regarding the pressor fraction, two pressor peptides having similar electrophoretic migrations (21 cm) but different chromatographic migrations (6–8 cm for lysine vasopressin, 10–12 cm for the new hormone) could be isolated. Spots, detected with dilute ninhydrin, were eluted with 0.1 M acetic acid. The yields in pressor activity were 27–36% for lysine vasopressin and 7–11% for the new hormone. Performic acid-oxidized or mercaptoethanol-added samples were subjected to hydrolysis (6 M HCl, 48 h, 105 °C *in vacuo*) and analysed with a Spinco 120B automatic analyser fitted with a high-sensitivity cell⁶. Results for the new hormone are given in Table 1. About 70 nmol of lysine vasopressin and 30–40 nmol of phenypressin were recovered from about 40 mg of acetone posterior pituitary powder.

For sequential studies, products purified from 14 glands of red kangaroo on one hand, and from 33 glands of tammar on the other, were used. In this case, spots were not eluted after chromatoelectrophoresis but Edman recurrent degradation was directly applied by using the paper-strip adaptation described by Chauvet and Acher⁷. Identification of phenylthiohydantoin (PTH) amino acids was carried out by thin-layer chromatography according to Edman and Henschen⁸. Spots were located by fluorescence and by reaction with ninhydrin which often gives characteristic colours⁸. About 100 nmol of pig lysine vasopressin and 50 nmol of ox arginine vasopressin were subjected, for comparison, to chromatoelectrophoresis and Edman degradation in the same conditions.

In the new hormone, Phe, Phe, Gln, Asn and Pro were identified in positions 2, 3, 4, 5 and 7 (Cys in positions 1 and 6 could not be seen because of the disulphide bridge). Comparison with arginine vasopressin or lysine vasopressin clearly showed PTH-Tyr in position 2 (lemon-yellow coloration) for these hormones instead of PTH-Phe (violet coloration after ninhydrin) for the new hormone. In contrast, PTH-Phe was found in position 3 for the three peptides. To characterize the residues in position 8 and 9 in the new hormone, after the eighth cleavage material was eluted from the paper strip with 0.1 M acetic acid and subjected to paper electrophoresis (0.1 M pyridine acetate buffer at pH 3.7, 50 V cm⁻¹, 35 min). PTH-Arg could be identified by a modified Sakaguchi reaction⁹ (8.6-cm migration) and glycineamide with ninhydrin (20-cm migration). The two half-cystines, found as cysteic acid residues in performic acid-oxidized sample, were assigned to positions 1 and 6. A complete

Table 2 Amino acid sequence of phenypressin

Phenypressin	¹ Cys- ² Phe- ³ Phe- ⁴ Gln- ⁵ Asn- ⁶ Cys- ⁷ Pro- ⁸ Arg- ⁹ Gly (NH ₂)
Arginine vasopressin	¹ Cys- ² Tyr- ³ Phe- ⁴ Gln- ⁵ Asn- ⁶ Cys- ⁷ Pro- ⁸ Arg- ⁹ Gly (NH ₂)
Lysine vasopressin	¹ Cys- ² Tyr- ³ Phe- ⁴ Gln- ⁵ Asn- ⁶ Cys- ⁷ Pro- ⁸ Lys- ⁹ Gly (NH ₂)

sequence is proposed for the new hormone in Table 2. We suggest the name *phenypressin* because of the substitution by phenylalanine of the second residue of arginine vasopressin, the classical pressor hormone of mammals⁴. On the other hand the biological properties resemble those of vasopressins.

Phe²-Arg⁸-vasopressin was synthesized, 18 years ago, by Huguenin and Boissonnas¹⁰. They compared the pharmacological properties of this analogue with those of arginine vasopressin. The substitution reduces the rat antidiuretic activity per μ mol from 435 to 375 U and the rat blood pressure activity from 435 to 130 U. The intrinsic oxytocic activity is dramatically reduced from 17 to 0.2 U. From a limited number of measures, the rat blood pressure activity of natural phenypressin was found to range from 130 to 200 U, value in accordance with that reported for the synthetic peptide. Because of the small amount of material available and its very weak oxytocic activity, a reliable value could not be determined for this activity.

Examination by ion-exchange microchromatography¹ of six individual glands of red kangaroo collected either in Western Australia or in New South Wales, and of three individual glands of tammar, systematically revealed two pressor hormones. Duality was also found in individual glands of three other macropodid species (grey kangaroo, euro, quokka) and of two species belonging to the American family of Didelphidae (South American opossums)³. Apparently vasopressin duplication is current among marsupials. However, whether the pressor hormones of Didelphidae are identical with those of Macropodidae still needs clarification. Lysine vasopressin and phenypressin, characterized in two macropodid species, differ by two amino acid residues in positions 2 and 8. They are likely products of two distinct structural genes rather than two allelic forms of a single gene. Vasopressin dimorphism has previously been reported for some pig-like species (peccary, warthog, hippopotamus) without individual characterization^{11,12}. It was assumed that the placental pressor hormones, arginine vasopressin and lysine vasopressin, were present, and because both hormones were not observed in all the glands examined, dimorphism was explained by heterozygosity¹¹.

Egg-laying prototherians are supposed to be the closest present-day mammals to the ancestral mammalian reptiles. The neurohypophyseal hormones of an Australian prototherian, echidna, have been isolated and conventional mammalian peptides, oxytocin and arginine vasopressin, have been characterized¹³. All the non-mammalian tetrapods examined to date, including four reptile species, have mesotocin and arginine vasotocin⁴ so that the change of arginine vasotocin into arginine vasopressin seems to have occurred early in mammalian evolution. In *Metatheria*, a duplication of the vasopressin gene occurred later and subsequent mutations might have produced lysine vasopressin on one hand and phenypressin on the other. Because neurohypophyseal hormones are probably fragments of larger precursors in which they are bound to neurophysins¹⁴ and because two types of neurophysins have been found in placental mammals¹⁵, it will be of interest to establish whether the additional marsupial hormone is accompanied by an additional neurophysin.

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Table 1 Amino acid composition of red kangaroo and tammar phenypressins

Amino acid	Red kangaroo (13 nmol)	Tammar (15 nmol)	Phenypressin (theoretical values)
Arg	0.88	0.93	1
Asp	1.00	1.00	1
Glu	1.06	1.08	1
Pro	1.03	0.71	1
Gly	1.15	1.22	1
Tyr	—	0.10	—
Phe	1.77	2.10	2
Cys*	1.18	1.33	2

Values are in molar ratios, using aspartic acid as reference.

* Half-cystines are determined as cysteic acid on a separate performic acid-oxidized sample. A partial destruction of cysteic acid is observed when hydrolysis is carried out with paper-eluted peptide.

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HCG stimulation of testicular LHRH-like activity

Richard M. Sharpe & Hamish M. Fraser

MRC Reproductive Biology Unit, Centre for Reproductive Biology, 37 Chalmers Street, Edinburgh EH3 9EW, UK

It is well established that the administration of high doses of luteinizing hormone (LH) or human chorionic gonadotropin (HCG) to animals can impair gonadal function in both the male and female¹. These effects can be duplicated by the administration of LH releasing hormone (LHRH) or its agonists to intact animals^{2–5}, including man⁶. Somewhat surprisingly, recent results have demonstrated that similar impairment of gonadal function by LHRH and its agonists can be achieved in the absence of the pituitary (for review see ref. 7), and the demonstration of specific, high-affinity binding sites for LHRH and its agonists on ovarian luteal cells⁸ and on testicular Leydig cells^{9–11}, could mean that LHRH-like factors are involved in the local regulation of the gonads. A further intriguing finding is the striking similarity between the direct, inhibitory effects of LHRH and LH/HCG on the gonads⁷; for example, both treatments reduce LH receptor numbers and the steroidogenic responsiveness of rat Leydig cells^{2,4,12,13}. We now offer a possible explanation for this similarity by demonstrating that HCG treatment stimulates the testicular production of a biologically active LHRH-like factor, suggesting that the inhibitory actions of HCG on the Leydig cell may be mediated by the local production of this factor.

We have utilized the receptor-binding ability of rat Leydig cells for LHRH and its agonists^{9–11} to establish a robust and sensitive assay for LHRH-like activity present in testicular interstitial fluid (IF) (Fig. 1). Using this assay system, negligible activity was detectable in IF from saline-injected (control) rats whereas clearly detectable amounts were present 16 h after injection of 100 IU HCG. Interstitial fluid from such animals gave parallel displacement curves to unlabelled LHRH agonist (Fig. 1), and stimulated the release of pituitary LH from hemipituitaries *in vitro* (Table 1), establishing that the material was biologically active. Other factors present in high amounts in the IF, such as HCG¹⁴ and testosterone¹⁵, did not contribute to this activity, as the addition of these hormones in large amounts (HCG up to 10^{-6} M, testosterone up to $10 \mu\text{g ml}^{-1}$) to the receptor assay was without significant effect. Again, the inclusion of IF from control and HCG-injected animals in the rat LH radioimmunoassay was without detectable effect, thus demonstrating that the LH measured in hemi-pituitary incubation medium was derived from the pituitary tissue and not from the added IF.

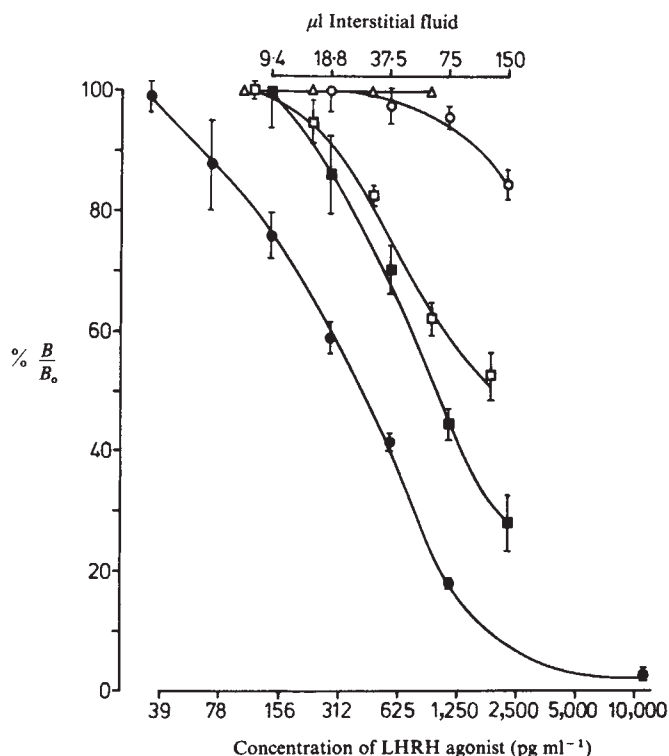


Fig. 1 Displacement of the binding of ^{125}I -LHRH agonist ((D-Ser-t-bu⁶, des-Gly-NH₂¹⁰) LHRH ethylamide) to dispersed Leydig cells by unlabelled LHRH agonist and testicular interstitial fluid from control and HCG-injected rats. Leydig cells were prepared by collagenase dispersion of decapsulated testes from adult (age ≥ 90 days) Liverpool/Hooded rats, using techniques described previously^{11,19}. The dispersed cells were resuspended in Krebs-Ringer bicarbonate (KRB) solution (pH 7.5) containing 0.2% bovine serum albumin (BSA; fraction V, Sigma) and $200 \mu\text{g ml}^{-1}$ Bacitracin (Sigma). The volume of resuspension was equivalent to 1 ml per original testis and, for assay, aliquots (0.2 ml) of this cell suspension were dispensed into 63×11 -mm polystyrene tubes. As determined by the staining of dispersed cells for 3β -hydroxysteroid dehydrogenase¹⁹, each 0.2-ml aliquot contained approximately 5×10^6 positively staining (Leydig) cells. The test materials or unlabelled LHRH agonist were added in a volume of 0.15 ml and ^{125}I -LHRH agonist ($\sim 150,000$ c.p.m.) in a volume of 0.05 ml. Nonspecific binding was determined by parallel incubation in the presence of 10^{-6} M unlabelled LHRH agonist (see ref. 11 for details). All samples/standards were run in duplicate or triplicate and incubation was for 45 min at 21°C . Incubation was ended by placing tubes on ice and immediately diluting the incubate with 1 ml ice-cold medium. Tubes were then centrifuged for 15 min at $1,000g$ and radioactivity in the pellet measured in a γ counter. LHRH agonist was labelled with ^{125}I using lactoperoxidase²⁰ as described previously¹¹ and, as determined by self-displacement in the receptor assay, had a specific activity of 1,200 mCi per mg. In eight successive assays the mean ($\pm$ s.d.) binding of ^{125}I -LHRH agonist was $7.4 \pm 1.5\%$ of the total counts added, of which 32–40% was not displaceable by parallel incubation with 10^{-6} M unlabelled LHRH agonist, and was taken to represent the nonspecific binding; specific binding was $5.6 \pm 1.3\%$ of the total counts added. The sensitivity of the assay, defined as the concentration of unlabelled LHRH agonist required to suppress binding to 90% B/B_0 , was $48 \pm 16 \text{ pg ml}^{-1}$ ($n = 8$), and the intra-assay coefficient of variation was $\pm 5\%$ for standards and $\pm 8.4\%$ for interstitial fluid samples. Sprague-Dawley rats aged 70–80 days were injected subcutaneously with either 0.9% saline or 100 IU HCG (Chorulon) and killed with ether 16 h later. Testicular IF was collected as described previously^{14,15}, by allowing the fluid to percolate from the incised caudal end of the testis over a 16-h period at 4°C ; the fluid was then centrifuged for 5 min at $1,000g$ at 4°C and the IF from 8–12 rats per treatment group was pooled before assay. The displacement curves for two such pools of IF from independent experiments are detailed in the figure. ●, Unlabelled LHRH agonist (standard); Δ , $\circ$, interstitial fluid from saline-injected (control) rats; ■, □, interstitial fluid from HCG-injected rats. Results are plotted as the mean and range of duplicate determinations and, as determined by 2-factor analysis of variance (with replication), there was no evidence ($P > 0.05$) for non-parallelism of the displacement curves for standard and IF dilutions.

We have used the receptor assay to quantify the LHRH activity in IF at various times after HCG injection (Table 2). In only 2 out of 12 samples from control rats (expts 1 and 2, Table 2) was there detectable LHRH activity, whereas high levels were found in all six samples from rats 16 h after injection of 100 IU HCG; at other times after this treatment, the frequency and level of detectable LHRH activity did not differ from controls. This effect of HCG on LHRH activity in IF at 16 h after injection was dose dependent (expt 2, Table 2), and a low dose of HCG (6 IU) was without detectable effect. As found previously^{14,15}, injection of HCG also increased the amounts of IF in the testis at 8–24 h after injection (Table 2), as a result of increased capillary wall permeability¹⁶, and therefore calculation of the total amounts of LHRH activity in IF at 16 h after injection would exaggerate the difference from control levels.

Although we have shown that a factor present in IF from HCG-injected rats has LHRH-like activity *in vitro* at both the Leydig cell and pituitary levels, it seems most likely that any physiological effects of this factor are restricted to the testis. In support of this idea, we have preliminary evidence which suggests that the receptor-binding potency *in vitro* of this

Table 1 Stimulation of pituitary LH release *in vitro* by LHRH and testicular IF from control and HCG-injected rats

Test material added to incubation medium	% LH released by test hemi-pituitary
10 ng LHRH	635 ± 192*
1 ng LHRH	101 ± 34
0.1 ml control IF	118 ± 50
0.01 ml control IF	76 ± 25
0.1 ml HCG + 16 h IF	357 ± 68*
0.01 ml HCG + 16 h IF	91 ± 15

Hemi-pituitaries from Sprague-Dawley rats aged 55 days were incubated at 37 °C *in vitro* for 4 h as described previously²¹. One-half of each pituitary was incubated in buffer alone (Krebs Ringer + 1 mg ml⁻¹ glucose + 0.2% bovine serum albumin (BSA)) while the contralateral hemi-pituitary was incubated in the presence of one of the test materials listed. The incubation volume was 0.5 ml. LH in the incubation medium was determined by radioimmunoassay²². LH released in the presence of the test material is expressed as a % of that released by the (control) contralateral hemi-pituitary. For the two test materials which stimulated LH release, the basal and test-stimulated LH release (mean ± s.d.) were as follows—10 ng LHRH: basal, 2.45 ± 0.25 µg NIAMDD rat LH-RP-1 per hemi-pituitary; stimulated, 15.35 ± 4.68 µg; 0.1 ml HCG + 16 h IF: basal, 2.78 ± 1.05 µg; stimulated, 9.38 ± 1.59 µg. Results are the mean ± s.d. for three incubations.

* $P < 0.01$ (paired *t*-test comparison of control and test-stimulated LH release).

Table 2 The relationship between the dose of HCG injected and the time after injection on the levels of LHRH-active material in testicular interstitial fluid

Expt	Treatment	Hours after injection	No. of animals	Interstitial fluid (µl per paired testes)	LHRH activity in IF (ng equiv. of LHRH agonist per ml)	
					Detectable/undetectable	Mean ± s.d.*
1	Saline 100 IU HCG	16	6	145 ± 19	1/5	1.24 ± 0.6
		2	4	123 ± 30	0/4	<1
		8	4	193 ± 21†	2/2	1.24 ± 0.2
		16	6	229 ± 44‡	6/0	5.48 ± 2.3‡
		24	4	213 ± 40†	0/4	<1
		40	4	130 ± 25	1/3	2.05 ± 1.8
2	Saline 150 IU HCG 30 IU HCG 6 IU HCG	16	6	107 ± 16	1/5	1.28 ± 0.7
		16	4	160 ± 7§	4/0	8.49 ± 4.0‡
		16	4	160 ± 14‡	4/0	5.29 ± 1.8‡
		16	4	138 ± 26	1/3	1.36 ± 0.6

Treatment of animals and collection of IF were as described in Fig. 1 legend, except that IF from individual animals was kept separate and not pooled. All the above samples were assayed together in 0.05-ml amounts in a single receptor assay, and the concentration equivalent of LHRH agonist in each sample was computed after log-logit transformation of the data. All samples were assayed in duplicate except for three control fluids for which only a single assessment was possible. The limit of detection of the assay (the level corresponding to 90% B/B_0) was equivalent to 1 ng LHRH agonist per ml for a 0.05-ml sample.

* All undetectable samples (<1 ng ml⁻¹) were assigned a value of 1 ng ml⁻¹ to calculate the mean ± s.d. and assess statistical significance.

† $P < 0.02$; ‡ $P < 0.01$; § $P < 0.001$, in comparison with respective control group (Student's *t*-test).

LHRH-like factor is considerably lower at the pituitary than at the Leydig cell level (H.M.F. and R.M.S., unpublished data). This may mean that there are differences in the type of degradative enzymes within the pituitary and the Leydig cell or that there are differences between the structures of hypothalamic LHRH and the LHRH-active factor produced by the testis. In this respect, the latter may correspond to the LHRH-active factor recently isolated from ovarian follicular fluid^{17,18}, which possesses LHRH-like biological activity but is immunologically distinct from native LHRH.

In rats injected with high doses of HCG, impairment of the steroidogenic responsiveness of the Leydig cells and the loss of LH (HCG) receptors occur dramatically beyond 16 h after injection¹⁴. This time coincides precisely with the appearance of detectable levels of the LHRH-active factor in the interstitial fluid that bathes the Leydig cells. As LHRH agonists can themselves directly reduce LH receptor numbers and the steroidogenic responsiveness of the Leydig cells^{12,13}, it seems plausible that the inhibitory effects of HCG on the Leydig cell are mediated at least in part by the local production of this LHRH-active factor. Its origin is unknown but, as granulosa cells seem to be the site of production of an LHRH-active factor in the ovary¹⁸, possibly the corresponding cell type in the testis, the Sertoli cell, is the site of production of the LHRH-active factor which we have detected. Certainly, the possibility that gonadotropins may affect gonadal function through locally

produced LHRH-like substances opens exciting new dimensions for reproductive biology and its control.

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Gating charge immobilization and sodium current inactivation in internally perfused crayfish axons

R. P. Swenson Jr

Department of Physiology/G4, University of Pennsylvania, Philadelphia, Pennsylvania 19104

The sodium permeability in nerve is regulated by two gating processes, activation and inactivation¹. Their relationship to one another has been described as 'independent'¹ or 'coupled'^{2,3}. An independent model, the Hodgkin and Huxley formulation, makes two testable predictions regarding gating current. First, as inactivation is voltage sensitive, it requires its own voltage sensor and might be expected to generate its own gating current. Second, gating current associated with activation should be unaffected by inactivation. Although charge movement associated with sodium activation is well characterized⁴, no inactivation charge movement has been described. Instead, depolarizations long enough to produce sodium current inactivation were found to diminish the charge movement associated with channel closing⁵. Inactivation temporarily immobilized a substantial fraction of the gating charge. These observations suit a coupled model in which inactivation gains its voltage dependence by coupling to activation³, and inactivation may in turn influence activation. However, the relative time course of the development immobilization (τ_{immob}) was found to be the same³, the same in some voltage ranges⁶ and slower than that for inactivation (τ_{inact}). In crayfish axons inactivation is roughly five times faster than in squid, providing an interesting opportunity to compare inactivation and immobilization. Further, inactivation is so rapid that inactivation gating current should be easily visible. No inactivation charge movement was found and τ_{immob} equals τ_{inact} .

Experiments were performed on internally perfused crayfish giant axons using an axial wire for voltage clamping the nerve. The solutions were prepared so that external sodium was the only permeant ion and gating currents were measured in the presence of tetrodotoxin (TTX). The relative time courses of inactivation in crayfish and squid nerve are compared in Fig. 1. The lower traces show that the sodium current (I_{Na}) decays with a time constant (τ_h) of 2.5 ms in squid and more quickly in crayfish, 0.45 ms at the same temperature and voltage. The upper traces in the figure show gating current recorded at the same voltage in the presence of TTX. The rapid time course of inactivation in crayfish should make it easy to resolve an additional component in the gating current due to inactivation, yet no such current is detectable at any potential.

Immobilization can be measured by integrating the gating current tails as the channels close at pulse termination (Q_{OFF}) and comparing them with the activation charge movement (Q_{ON}). The OFF gating currents diminish in both amplitude and area as the duration of the pulse was increased as shown in Fig. 2. After a depolarization of 10 ms (not shown), the OFF/ON ratio was 0.2; that is, 80% of the gating charge was temporarily immobilized and returned to its resting position too slowly to detect.

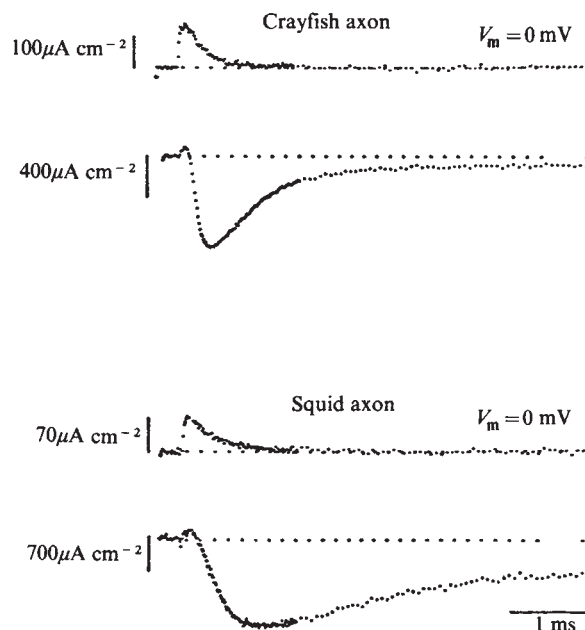


Fig. 1 Sodium and gating currents from crayfish and squid giant axon. Depolarizations to 0 mV from holding potentials of -80 mV (crayfish) and -70 mV (squid) elicit sodium currents of dramatically different time courses (lower traces). The ON gating currents (upper traces), are similar from the two nerves, and in neither is there a component with the time course of inactivation. Leakage and capacitive currents were subtracted using P/4 technique⁵ from both I_{Na} and I_g records, and I_g represents the addition of 10 records. Crayfish currents were recorded with 70 mM NaCl, 140 mM Trizma (pH 7.0), and 20 mM CaCl_2 externally and 60 mM CsF, 60 mM $\text{Cs}_3\text{Citrate}$, and 120 mM mannitol internally. 10^{-7} M TTX was added externally to record I_g (axon Fe010Z). Squid currents were recorded in 112 mM NaCl, 378 mM Trizma (pH 7.0), 50 mM CaCl_2 externally and 150 tetramethylammonium (TMA) glutamate, 50 mM TMAF, and 650 mM sucrose internally (axon AU169Z). Temperature, 8°C .

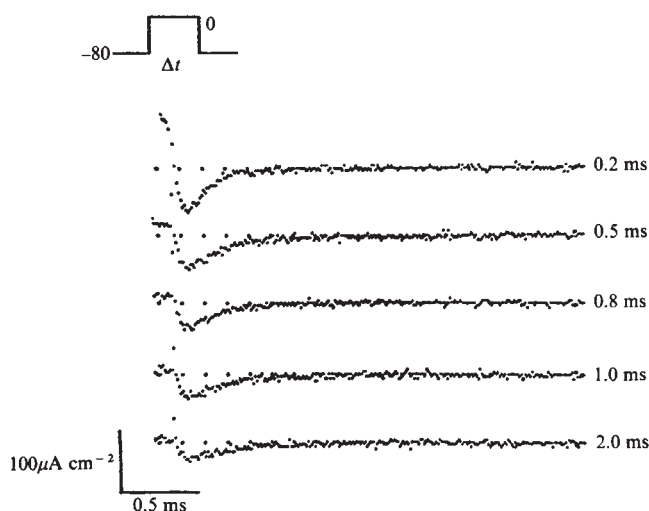


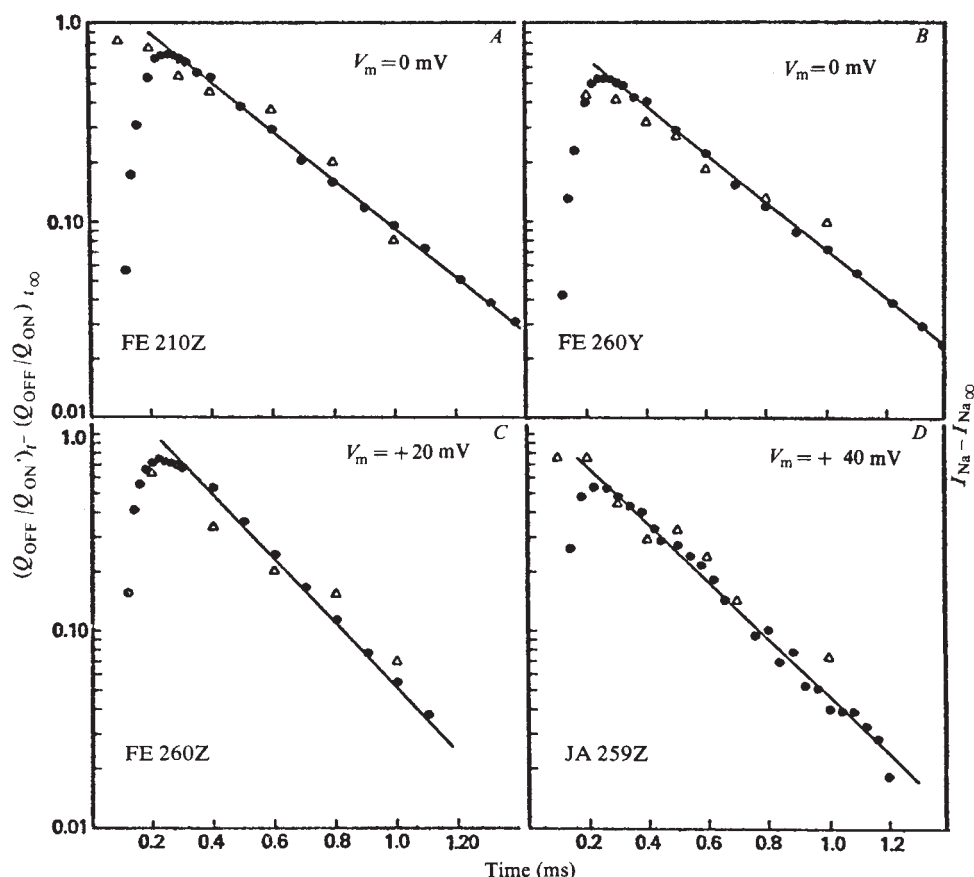
Fig. 2 OFF gating currents recorded following depolarization to 0 mV in crayfish axon. On returning to the holding potential of -80 mV after depolarizations of 0.2, 0.5, 0.8, 1.0 and 2.0 ms, the integrated areas of the OFF gating currents (Q_{OFF}) were 828, 815, 594, 590, and 534 $\text{e}^- \mu\text{m}^{-2}$, respectively. Q_{ON} was 1889 $\text{e}^- \mu\text{m}^{-2}$ for the 2-ms pulse. The external and internal solutions were the same as in Fig. 1. Temperature, 8°C . Axon Fe260Y.

Table 1 Comparison of the time constants at 8°C of sodium current inactivation (τ_h and τ_{tails}) and gating current immobilization (τ_{immob})

Potential	$\tau_h(n)$	$\tau_{\text{tails}}(n)$	$\tau_{\text{immob}}(n)$
-20 mV	0.55 ± 0.01 (4)	—	—
0 mV	0.46 ± 0.01 (4)	0.44 (1)	0.45 ± 0.04 (2)
$+20$ mV	0.39 ± 0.02 (3)	—	0.39 (1)
$+40$ mV	0.36 ± 0.01 (3)	0.31 (1)	0.38 (1)

n, Number of readings.

Fig. 3 Comparison of the rates of inactivation of the sodium current (●) and immobilization of the gating current (△) at 0, +20, and +40 mV in crayfish axon. $(I_{Na} - I_{Na\infty})$ is plotted where $I_{Na\infty}$ is the steady state sodium current. Axon Fe010Z. The sodium current in *a* and *b* is identical but slightly compressed on the vertical axis in *b*. Solutions as in Figs 1 and 2 without TTX. Peak amplitudes of the sodium currents were 0.84, 0.43, and 0.16 mA/cm² for 0, +20, and +40 mV, respectively. Superimposed on the sodium currents is the degree of immobilization (△) from four different axons measured from the area of the OFF gating currents after varying duration pulses. The OFF areas are plotted as the ratio of the integrated OFF gating current to ON gating current, $(Q_{OFF}/Q_{ON})_t$, minus a baseline correction, $(Q_{OFF}/Q_{ON})_{t\infty}$. t_{∞} was 3.0 or 10.0 ms. *a*, Axon Fe210Z; *b*, Fe260Y; *c*, Fe260Z; and *d*, JA259Z. Solutions were the same as in Figs 1 and 2 with TTX. Temperature, 8 °C in all cases.



The temporal correlation between inactivation and immobilization is exemplified in Fig. 3, where sodium currents from one experiment are plotted on a semilogarithmic scale together with the OFF/ON ratio from other experiments. To allow for incomplete ON charge movement following brief pulses (0.5 ms), the OFF charge movement is normalized against the ON charge movement at various times $[(Q_{OFF}/Q_{ON})_t]$ and the baseline $[(Q_{OFF}/Q_{ON})_{t\infty}]$ was subtracted. Four experiments are shown, two at 0 mV, and one each at +20 and +40 mV. In all four, inactivation and immobilization agree closely in time course.

Table 1 summarizes the measurements of the time constant of immobilization (τ_{immob}), the time constant of inactivation of the sodium current measured from falling phase of the sodium current (τ_h), and the time constant of inactivation of the sodium current measured from sodium tail currents (τ_{tails}) at several potentials. The falling phase of the sodium current was well fitted by a single exponential. The two methods of measuring the rate in inactivation agree with one another, and were practically identical to τ_{immob} .

Although crayfish gating currents differ slightly in form from those measured in squid axons, the relatively fast rate of inactivation has allowed further evaluation of the relationship between the processes of inactivation and immobilization. The measurements reported here conform closely to the predictions of a coupled model: no inactivation gating current is visible in spite of the rapid time course of inactivation, and the time courses of inactivation and immobilization are identical. From these observations and others^{3,6} it seems that inactivation of the sodium current and immobilization of the gating charge are in fact manifestations of the same underlying event. Following reorganization of the charged particles in the nerve which permits sodium conductance, a state develops which is characterized by both diminished conductance and a decreased ability of the charged particles to reprime. The development of this state can be followed either as inactivation of sodium current or immobilization of sodium gating current.

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Are only α_2 -adrenergic receptors present in bovine retina?

Helmut Bittiger, Jakob Heid & Niklaus Wigger

Research Department of the Pharmaceuticals Division of Ciba-Geigy Ltd, CH-4002 Basle, Switzerland

The retina represents a part of the central nervous system (CNS) with a well studied, geometrically defined structure and a specialized function—the processing of light signals¹. Neurotransmitters such as glutamate, aspartate, glycine^{2,4}, γ -aminobutyric acid (GABA), dopamine and acetylcholine⁵⁻⁹ are considered to be involved in the neuronal activity of the retina. Receptors for acetylcholine^{10,11}, GABA¹², dopamine¹³ and benzodiazepines^{14,15} have also been demonstrated. Thus, the retina can be considered as a model for the study of neuronal processing in general, in which the input, light signals, can be selected and regulated in a defined way. We report here that α -adrenergic receptors in the bovine retina have been characterized using radioreceptor assays. ³H-phentolamine (an α_1 - and α_2 -antagonist), ³H-clonidine (a preferential α_2 -agonist) and ³H-WB 4101 [(2,6-dimethoxyphenoxyethyl)aminomethyl-1,4-benzodioxane, an α_1 -antagonist¹⁶] were used as radioligands,

and WB 4101 and prazosin¹⁷ (α_1 -antagonists), tolazine (an α_2 -antagonist)¹⁸, yohimbine and its stereoisomers rauwolfscine (preference for α_2 -receptors) and corynanthine (preference for α_1 -receptors)^{19,20}, were used as inhibitors of radioligand binding. Only α_2 -adrenergic receptors^{21,22} were found.

Figure 1a gives the saturation curve and corresponding Scatchard plot for ³H-phenolamine binding. The dissociation constant (K_D) was found to be 3.4×10^{-10} M and the number of binding sites 437 fmol per mg protein. Nonspecific binding was defined in the presence of 10^{-6} M phentolamine and was only 5–10% of total binding. Association and dissociation were remarkably rapid in view of the low K_D values. At 20 °C and a concentration of 0.5 nM ³H-phenolamine, half-saturation of binding was achieved within 2.5 min, and the half life of dissociation was 2.5 min (data not shown). ³H-clonidine showed nearly identical binding characteristics (Fig. 1b), with a dissociation constant of 3.2×10^{-10} M and 341 fmol binding sites per mg protein. Association and dissociation had half lives of 1.5–2 min for a fast component, but there were, in addition, slower components with about 5–10-min half lives, which contributed about 20% of the total binding sites (data not shown). The binding characteristics of ³H-WB 4101 are presented in Fig. 1c. Phentolamine, 10^{-6} M, was used to define nonspecific binding. The dissociation constant of 3.42 nM was

higher but the number of binding sites of 378 fmol per mg protein comparable to those obtained with ³H-phenolamine and ³H-clonidine. The ratio of specific to nonspecific binding was much less favourable than with ³H-clonidine and ³H-phenolamine. Selected compounds were tested for their effects on ³H-phenolamine, ³H-clonidine and ³H-WB 4101 binding in the retina and on ³H-WB 4101 binding in the rat brain (see Table 1). The latter values are from own experiments or the literature²³. Data from rat brain are usually similar to those from bovine brain^{23,24}. Surprisingly, all compounds studied in the retina inhibited with about equal potency in all three radioreceptor assays. The somewhat lower IC_{50} values in the ³H-WB 4101 assay are probably due to the fact that the ³H-ligand concentration of 1 nM was below the K_D of 3.42 nM, whereas those of ³H-phenolamine and ³H-clonidine were slightly above. Only agonists like noradrenaline, dopamine and clonidine were more potent in the ³H-clonidine assay.

The order of inhibitory potencies of yohimbine and its stereoisomers in the retina was found to be rauwolfscine > yohimbine > corynanthine, typical of α_2 -receptors²⁰. In the brain ³H-WB 4101 assay, however (Table 1, last column), the reverse order, typical of α_1 -receptors, was found²⁰. Consistent with these findings, tolazoline, interacting preferentially with α_2 -receptors, inhibited 140 times more potently in the retina

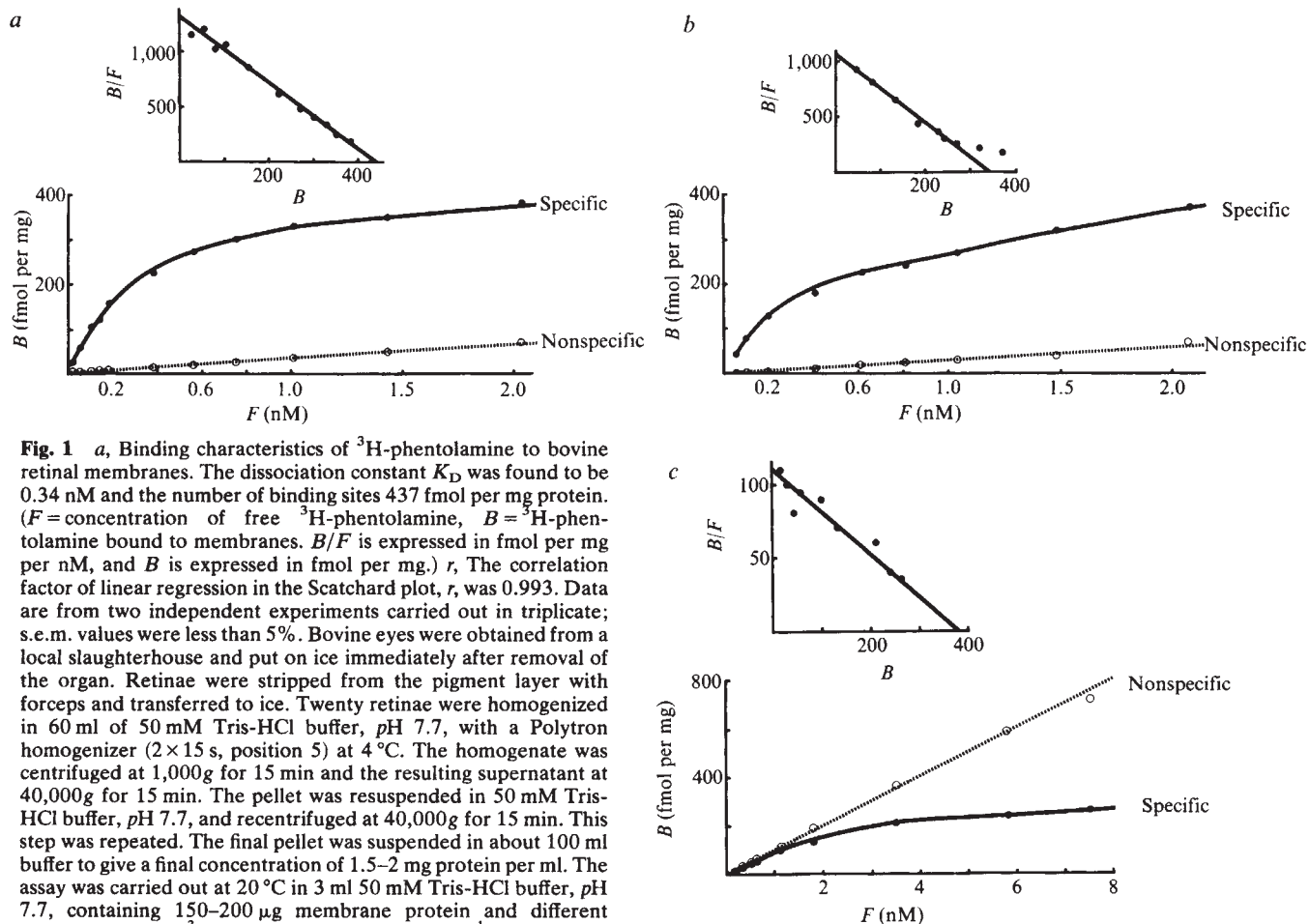


Fig. 1 a, Binding characteristics of ³H-phenolamine to bovine retinal membranes. The dissociation constant K_D was found to be 0.34 nM and the number of binding sites 437 fmol per mg protein. (F = concentration of free ³H-phenolamine, B = ³H-phenolamine bound to membranes. B/F is expressed in fmol per mg per nM, and B is expressed in fmol per mg.) r , The correlation factor of linear regression in the Scatchard plot, r , was 0.993. Data are from two independent experiments carried out in triplicate; s.e.m. values were less than 5%. Bovine eyes were obtained from a local slaughterhouse and put on ice immediately after removal of the organ. Retinae were stripped from the pigment layer with forceps and transferred to ice. Twenty retinae were homogenized in 60 ml of 50 mM Tris-HCl buffer, pH 7.7, with a Polytron homogenizer (2×15 s, position 5) at 4 °C. The homogenate was centrifuged at 1,000g for 15 min and the resulting supernatant at 40,000g for 15 min. The pellet was resuspended in 50 mM Tris-HCl buffer, pH 7.7, and recentrifuged at 40,000g for 15 min. This step was repeated. The final pellet was suspended in about 100 ml buffer to give a final concentration of 1.5–2 mg protein per ml. The assay was carried out at 20 °C in 3 ml 50 mM Tris-HCl buffer, pH 7.7, containing 150–200 μ g membrane protein and different concentrations of ³H-phenolamine (20 Ci mmol⁻¹, prepared in the Ciba-Geigy radioisotope laboratories by halogen-tritium exchange in the aromatic ring, purity >98% as shown by pressure liquid chromatography and isotope dilution analysis). Incubation was for 15 min and was terminated by rapid filtration through Whatman glass fibre filters GF/B and three washes with 4 ml ice-cold buffer. Nonspecific binding was defined in the presence of 10^{-6} M phentolamine. Filter-bound material was digested in Soluene (Packard) and radioactivity was counted by addition of scintillator (AquaSol, NEN) in an Intertechnique liquid scintillation counter at an efficiency of 42%. b, Binding characteristics of ³H-clonidine (NEN, 22.2 Ci mmol⁻¹) to bovine retinal membranes. The dissociation constant K_D was found to be 0.32 nM, the number of binding sites 341 fmol per mg protein and r 0.992. The experiments were performed as described in a. Nonspecific binding was defined in the presence of 10^{-6} M clonidine. c, Binding of ³H-WB 4101 (NEN, 24.2 Ci mmol⁻¹) to bovine retinal membranes. Nonspecific binding was defined in the presence of 10^{-6} M phentolamine. The dissociation constant was found to be 3.42 nM, the number of binding sites 378 fmol per mg protein, comparable to those of ³H-phenolamine and ³H-clonidine, and r 0.948. To reduce nonspecific binding, retinae were osmotically shocked during the first homogenization in 1 mM Tris-HCl buffer and filtration was through GF/C glass fibre filters. Otherwise the procedure was as described in a.

Table 1 Radioligand inhibition of drug binding to retinal and brain membranes

Compound	Retina			Brain
	³ H-phentolamine IC ₅₀ (M)	³ H-clonidine IC ₅₀ (M)	³ H-WB 4101 IC ₅₀ (M)	³ H-WB 4101 K _i or IC ₅₀ (M)
Phentolamine	1.0 × 10 ⁻⁹	1.0 × 10 ⁻⁹	8.0 × 10 ⁻¹⁰	1.0 × 10 ⁻⁸
Clonidine	6.4 × 10 ⁻⁹	1.4 × 10 ⁻⁹	3.6 × 10 ⁻⁹	4.3 × 10 ⁻⁷ (ref. 23)
WB 4101	2.0 × 10 ⁻⁸	2.0 × 10 ⁻⁸	1.6 × 10 ⁻⁸	6.0 × 10 ⁻¹⁰ (ref. 23)
Prazosin	5.8 × 10 ⁻⁶	7.2 × 10 ⁻⁶	2.0 × 10 ⁻⁶	8.0 × 10 ⁻¹⁰
Tolazoline	5.0 × 10 ⁻⁸	3.6 × 10 ⁻⁸	6.4 × 10 ⁻⁸	8.0 × 10 ⁻⁶
Yohimbine	5.0 × 10 ⁻⁸	5.6 × 10 ⁻⁸	3.6 × 10 ⁻⁸	4.8 × 10 ⁻⁷
Rauwolscine	1.0 × 10 ⁻⁸	1.6 × 10 ⁻⁸	5.6 × 10 ⁻⁹	1.0 × 10 ⁻⁵
Corynanthine	1.1 × 10 ⁻⁶	1.1 × 10 ⁻⁶	6.4 × 10 ⁻⁷	3.0 × 10 ⁻⁷
Noradrenaline	2.6 × 10 ⁻⁷	5.0 × 10 ⁻⁸	2.2 × 10 ⁻⁷	1.0 × 10 ⁻⁶ (ref. 23)
Dopamine	2.0 × 10 ⁻⁶	6.0 × 10 ⁻⁷	1.6 × 10 ⁻⁶	4.4 × 10 ⁻⁵ (ref. 23)
Propranolol	>10 ⁻⁵	>10 ⁻⁵		6.9 × 10 ⁻⁶ (ref. 23)

Assays were carried out as described in Fig. 1 legend. Drugs were added to the incubation medium at the same time as the radioligands. Concentrations of ³H-phentolamine and ³H-clonidine were 0.5 nM (slightly above K_D) and that of ³H-WB 4101 nM (below K_D). IC₅₀ values were determined graphically from 8–10 different concentrations of drugs, each point performed in triplicate. Brain membranes were prepared as described in the literature²³ and the radioreceptor assays were carried out as described for retinal membranes.

than in the brain, whereas WB 4101 and prazosin, α₁-antagonists, were respectively, 30 and up to 2,000 times, more potent in the brain. Thus, the retinal α-receptors labelled by ³H-phentolamine, ³H-clonidine and ³H-WB 4101 seem to be of the α₂-type and the brain receptors labelled by ³H-WB 4101 of the α₁-type.

The retinal α-receptors seem to differ from α-receptors in all tissues or cells so far studied with these three radioligands. The K_D for ³H-clonidine binding (0.32 nM) is much lower than that found in brain (5.6 nM)²³, although recently by using a special technique, a minority of high affinity ³H-clonidine binding sites (0.5 nM) has been detected in brain²⁶. ³H-WB 4101, on the other hand, binds with much lower affinity to retinal (3.42 nM) than to brain membranes (0.35 nM)²³ and seems to label α₂-receptors in the retina and α₁-receptors in the brain. As seen in Table 1, prazosin seems to be more specific for α₁-receptors than is WB 4101. ³H-phentolamine has a 35 times higher affinity for retinal α-receptors (K_D = 0.34 nM) than has been reported for α-receptors on human platelets (K_D = 12 nM)²⁵. We have found that ³H-phentolamine is not a suitable ligand for studying α-receptors in brain membranes, as very little specific binding can be detected. This may be due to the approximately 30 times higher dissociation constant in brain, as estimated from the inhibition constants in the ³H-clonidine and ³H-WB 4101 assays, which were of the order of 10⁻⁸ M (Table 1).

Retinal α-receptors seem to be peculiar in another respect. The relative inhibitory potencies of the agonists clonidine, dopamine and noradrenaline differ at most by a factor of five in the three radioreceptor assays. In the brain, however, these agonists are 60–180 times more potent in the ³H-clonidine assay than in the ³H-WB 4101 assay²³.

Receptors of the α₂-type have also been described on post-synaptic organs^{27,28}, so it cannot be concluded that the retinal α₂-receptors are functionally presynaptic. In marked contrast to the large number of α-receptors present in the retina, the concentration of noradrenaline is very low⁸. On the other hand, dopamine is found in retinæ of different species⁸; binding to the retinal α₂-receptors and a functional interaction between the two cannot be excluded. Our studies do not reveal whether retinal α-receptors are functional or simply phylogenetic remnants.

In conclusion, the retina seems to be specific not only in geometry and function, but also in terms of neurotransmitter receptors. The specialization of α₂-receptors in the retina may have important consequences not only for the mechanism of signal processing in this part of the CNS, but also in a minority of receptors²⁶ for neuronal processing in other parts of the CNS.

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Retrograde axonal transport of target tissue-derived macromolecules

I. A. Hendry & C. E. Hill

Department of Pharmacology, John Curtin School of Medical Research, Australian National University, PO Box 334, Canberra, Australia

Neurones depend on contact with their target tissues for survival and subsequent development^{1–4}. The protein, nerve growth factor (NGF), can be selectively taken up by sympathetic nerve terminals and reaches the neuronal perikaryon by a process of retrograde intra-axonal transport⁵, suggesting that its role *in vivo* is to act as a target tissue-derived trophic factor⁶. The development of the neurones of the chick ciliary ganglion requires the presence of structures derived from the optic cup⁴. Several studies *in vitro* have shown that media conditioned by non-neuronal cells contain factors that result in the survival of neurones from ciliary ganglia^{7–11}. In particular, chick embryo iris, ciliary body and choroid contained large amounts of these factors indicating the presence of a target tissue-derived trophic factor for the cholinergic ciliary ganglion¹². This study demonstrates that neurones of the ciliary ganglion accumulate, by retrograde intra-axonal transport, proteins synthesized and released by optic tissues in culture.

The iris, ciliary body and choroid were dissected aseptically from 19-day-old chicken embryos, and material from one eye placed in 2 ml of Eagle's medium containing Hanks salts, 50 mM HEPES and 100 U ml⁻¹ penicillin, and incubated at 37°C in humid air. The ability of the conditioned medium to support parasympathetic neurones from 8-day-old chick embryos was assessed after 24 h in culture as previously described¹³, and one trophic unit was defined by the volume of conditioned medium required to obtain a 50% neuronal survival^{11,13}.

The amount of neurotrophic activity in the medium increased with time and reached a level of 80 U ml⁻¹ after 5 days. Parallel cultures containing ³H-labelled amino acid mixture (Amersham, TRK 440) at 0.5 mCi ml⁻¹ in place of the corresponding unlabelled amino acids showed a large accumulation of labelled protein in the medium; at 5 days 9% of the total radioactivity was precipitated by 5% trichloroacetic acid. The radioactive medium was dialysed against 154 mM NaCl to lower the labelled amino acid content and then 25-μl aliquots were injected into the right eye of either 1-day-old or 2-week-old chicks. After 16 h, right and left ciliary ganglia were removed, homogenized separately in 100 μl distilled water, and 20-μl samples counted in a scintillation counter. There was a significant accumulation of radiolabel in the ganglion on the injected side compared to the non-injected side indicating the retrograde axonal transport of material from the conditioned medium (Table 1). This accumulation of radioactivity was

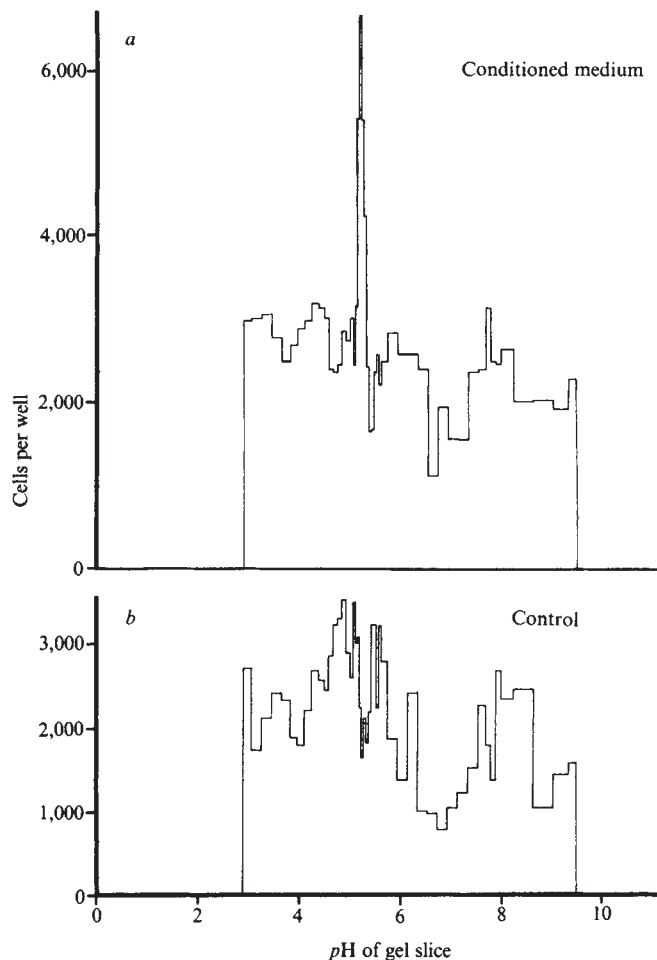


Fig. 2 Survival of parasympathetic neurones from 8-day-old chick embryos after 24 h in the presence of dialysed eluates of 1-mm slices from polyacrylamide isoelectric focusing gels. *a*, Medium conditioned by optic tissues. *b*, Control gel. The number of surviving neurones per 2-cm² well is plotted against the pH of each gel slice. In the absence of conditioned medium or gel eluates 900–1,500 cells per 2-cm² well survived.

unlikely to be due to diffusion from the injection site, as an injection of 25 times the amount of ³H-labelled amino acids in 154 mM NaCl did not result in a significant accumulation of radioactivity on the injected side (Table 1). This also demonstrates that the effect seen with the conditioned medium was not due to transport of residual labelled amino acids in the medium.

To determine the selectivity of the retrograde transport, the macromolecules in the conditioned medium and in the pooled ciliary ganglion homogenates from the injected and non-injected sides were separated by polyacrylamide gel isoelectric focusing using pH 3.5–10 ampholines (LKB). There were many labelled macromolecules in the conditioned medium (Fig. 1*a*) but only five major labelled species were seen in homogenates of ciliary ganglia from the injected sides of 2-week-old chicks (Fig. 1*b*). Thus the retrograde axonal transport was selective for comparatively few of the macromolecules released by the cultured target tissues. There were no major radioactive peaks detected in the homogenates of the ciliary ganglia from the non-injected sides (Fig. 1*c*).

The ability of the conditioned medium to support parasympathetic neurones was assessed after polyacrylamide gel isoelectric focusing to see if any of the transported species might have neurotrophic activity. Conditioned medium was dialysed for 6 h against distilled water and the gels run for 3 h at 0°C. Serial 1-mm slices were each eluted with 1 ml distilled water and the pH determined. The ampholines were then removed by

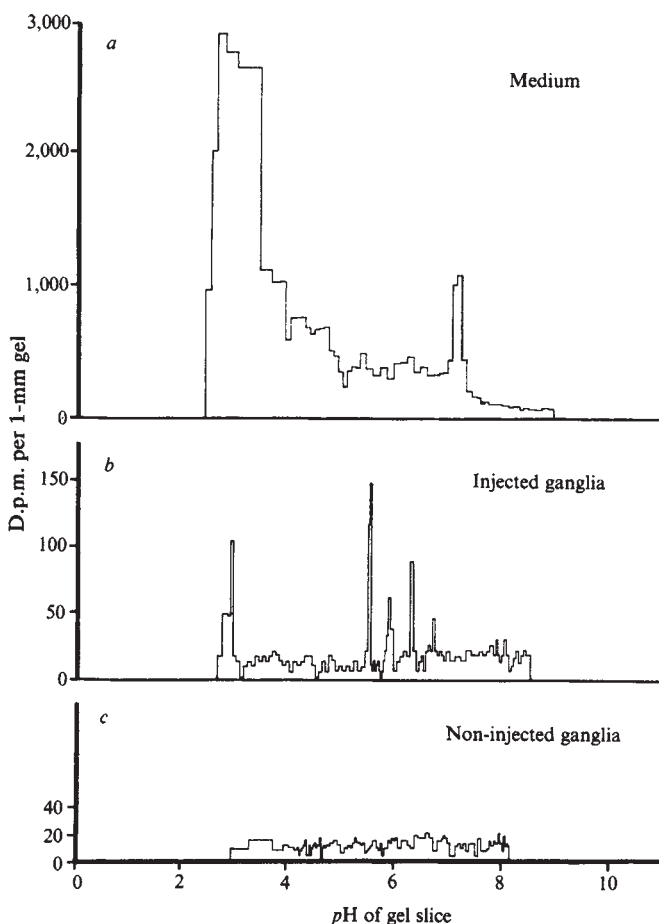


Fig. 1 Distribution of radioactivity after isoelectric focusing of: *a*, medium containing ³H-labelled amino acids and conditioned by a 5-day exposure to optic tissues from 19-day-old chick embryos. *b*, Pooled homogenates of right ciliary ganglia from six 2-week-old chicks injected in the right eye with this conditioned medium. *c*, Homogenates of left ciliary ganglia from the same animals. In each case gels were cut into 1-mm sections which were eluted with 1 ml distilled water for radioactivity and pH measurements. The radioactivity (d.p.m.) is plotted against the pH of each gel slice.

Table 1 Accumulation of radioactivity in chick ciliary ganglion after intraocular injection of radioactive conditioned medium or ^3H -labelled amino acids

	Age of injected chicks	Radioactivity (d.p.m. per ganglion)	
		Injected	Non-injected
Conditioned medium	1 day	2820 $\pm$ 630 (4)	15 $\pm$ 11 (4)
	2 week	6690 $\pm$ 645 (7)	90 $\pm$ 33 (7)
^3H -labelled amino acids	1 day	50 $\pm$ 10 (6)	33 $\pm$ 10 (6)
	2 week	9 $\pm$ 6 (6)	13 $\pm$ 5 (6)

The right eye was injected with 25 μl of either ^3H -labelled amino acids, 10 μCi or conditioned medium, 0.4 μCi . Sixteen hours later the right and left ciliary ganglia were removed and the radioactivity estimated. No. of ganglia in parentheses.

dialysis against 10 mM HEPES, pH 7.4, before the sample was mixed with medium for the determination of neurotrophic activity. Control gels were run similarly with distilled water in place of the conditioned medium. The component of the conditioned medium responsible for neuronal survival focused in the region of pH 5 (Fig. 2). One of the retrogradely transported radioactive proteins also focused in this region, suggesting that they may be the same.

In all neurones there must be a system for the retrograde transfer of information from the target cell to the innervating

neurone. This information transfer can be accomplished by target tissues synthesizing and releasing proteins which are taken up by nerve terminals and retrogradely transported to neuronal cell bodies resulting in appropriate stimulation of metabolic pathways. It is likely that at least one of the retrogradely transported species described here is a retrophin (the retrogradely transported target tissue-derived neurotrophic factor) for neurones of the ciliary ganglia.

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Transmitter release from frog motor nerve terminals depends on motor unit size

A. A. Herrera & A. D. Grinnell

Department of Physiology, Jerry Lewis Neuromuscular Research Center, Ahmanson Laboratory of Neurobiology, School of Medicine, University of California, Los Angeles, California 90024

It has been postulated that the success with which a motor nerve terminal competes for synaptic connections^{1,2} or the ability of an axon to maintain sprouts³ may depend on the support each terminal receives from its soma, presumably in the form of some substance(s) synthesized there. The support received by each terminal may in turn depend on the total number of terminals maintained by that soma, namely, motor unit size. We show here that when motor unit size is experimentally decreased, transmitter release from the terminals is markedly enhanced. This is consistent with the view that the extent of support from the soma may also influence the effectiveness of synaptic transmission.

Eight to 12 weeks before acute experiments, the left sartorius nerve of adult *Rana pipiens* (body length 4.5–6.5 cm, anaesthetized in 0.2% tricaine methanesulphonate) was crushed near its entrance to the muscle. A lengthwise cut was made through the sartorius to remove muscle fibres in its lateral half. Thus when the motor axons regenerated, they encountered and re-innervated approximately half the normal number of fibres. Although there was repair of damaged muscle fibres at the cut edge, there was no evidence of substantial muscle regeneration. Control sartorius muscles, where the nerve was crushed but the muscle left intact, and normal muscles from unoperated frogs were tested for comparison. Samples were compared with the Mann-Whitney *U*-test unless otherwise noted. All data are presented here as mean $\pm$ s.e.m.

The number of increments in twitch tension produced by slowly increasing or decreasing the strength of a stimulus to the nerve was not significantly different ($P < 0.05$, Kruskal-Wallis *H*-test) in experimental (9.7 ± 0.5 , $n = 14$), control (9.1 ± 0.9 , $n = 7$) and normal (8.2 ± 0.35 , $n = 13$) muscles suggesting that

muscles in both operated groups become re-innervated by the same number of motor axons. Maximal twitch tension in experimental muscles (10.7 ± 1.4 g, $n = 15$) averaged approximately two-thirds that of controls (16.3 ± 2.0 g, $n = 7$), reflecting the removal of a substantial number of muscle fibres. In both whole and halved re-innervated muscles, the distribution of motor unit sizes, relative to the maximum tension that could be evoked, was essentially the same as normal. Mean motor unit size, expressed as a percentage of maximal nerve evoked tension, was not significantly different in experimental (10.1%), control (11.0%) or normal (12.2%) muscles. These observations suggest that most or all motor axons participated in re-innervation and that the size of the peripheral field of each motoneurone in the experimental case was indeed reduced.

Synaptic strength was tested in two ways, using previously described techniques⁴. In each case the degree of stretch of the muscle, known to affect synaptic strength⁵, was carefully controlled. The first method, which estimates safety factors for junctional transmission, consisted of measuring the tension of twitches evoked by a single nerve stimulus while varying Ca^{2+} concentration in the Ringer's solution. When the Ca^{2+} concentration is lowered, twitch tension falls as transmission at the strongest junction on different fibres becomes sub-threshold⁴. At any particular low Ca^{2+} concentration, the extent to which tension has fallen is a measure of safety factors at junctions in that muscle. Using this approach, we compared safety factors in experimental, control and normal muscles by measuring the tension remaining after Ca^{2+} concentration was lowered from a normal value of 1.8 mM to 1.0 and 0.6 mM (Fig. 1). In Ringer containing 1 mM Ca^{2+} , tension in experimental muscles with reduced motor unit size fell only to 79% of the tension in normal Ringer. This was significantly more than the tension remaining in control or normal muscles (55% and 42% respectively, $P < 0.01$). Results from control and normal muscles were not significantly different ($P > 0.05$). In 0.6 mM Ca^{2+} , experimental muscle tension only fell to 22%, whereas control tension averaged 4% and normal tension 5%. Again, the differences between the experimental case and the other two cases were significant ($P < 0.02$).

The second method of testing synaptic strength was designed to determine if the increase in safety factor was due to a

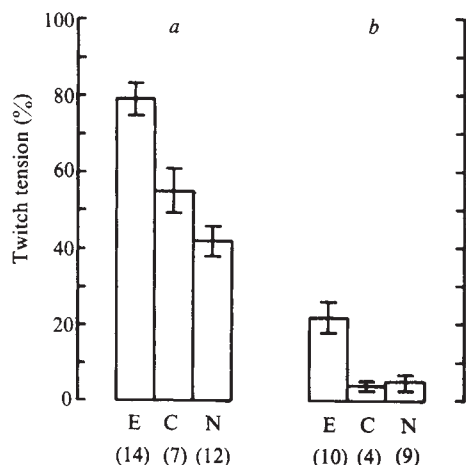


Fig. 1 The effect of lowered Ca^{2+} concentration (a, 1.0 and b, 0.6 mM) on twitch tension evoked by nerve stimulation. Tension is expressed as a percentage of the tension generated in normal Ringer containing 1.8 mM Ca^{2+} . At each concentration the mean, $2 \times \text{s.e.m.}$, and the number of muscles tested are shown for experimental (E, re-innervated half sartorius), control (C, re-innervated whole sartorius) and normal (N, unoperated sartorius) muscles. Experimental and control muscles were denervated by nerve crush 60–84 days earlier.

difference in the amount of transmitter release from nerve terminals in the experimental muscles. We measured end plate potential (e.p.p.) quantal content in Ringer containing 0.3 mM Ca^{2+} and 1 mM Mg^{2+} . For the operated muscles, junctions on regenerated muscle fibres in the area of the cut were not included in the quantal content analysis, nor were multiply innervated junctions, which were occasionally found but not at a frequency noticeably higher than in re-innervated whole muscle controls. As shown in Table 1, quantal content in muscles with reduced motor unit size was more than twice as great as that of control junctions (6.3 compared with 2.8) and three times greater than quantal content at normal junctions (2.1). The difference between experimental and control junctions was statistically significant ($P < 0.02$), but that between control and normal junctions was not. Miniature e.p.p. frequencies in the half muscles were also greater to approximately the same degree.

Because it has been shown that quantal content depends in part on nerve terminal size⁶, the junctions from which recordings were made were marked by injection of a dye into the muscle fibre. These fibres were then identified in the light microscope and stained with the nitro blue tetrazolium (NBT) nerve terminal stain⁷ and cholinesterase (ChE) stain⁸. Nerve terminal length was taken to be the sum of the individual lengths of NBT-stained processes located within the synaptic gutters outlined by ChE stain. As ChE activity persists for many weeks even in denervated gutters, we were able to observe that for both the re-innervated whole muscles and the undamaged fibres of the halved muscle, re-innervation occurred only at old endplate

sites. No NBT-stained terminals were found without ChE and ChE deposits always looked normal in shape and size, unlike ectopic synapses seen in foreign nerve re-innervated sartorius muscles (A.D.G., unpublished data) or *de novo* synapses on cutaneous pectoris muscle fragments⁹. We conclude, therefore, that few if any ectopic synapses were formed. Using identified terminals that had been studied electrophysiologically, quantal contents were normalized to nerve terminal length. As shown in Table 1, terminals in muscles with reduced motor unit size released 1.89 quanta per 100- μm nerve terminal length. This was significantly higher than release per unit length in control (0.46) and normal (0.45) terminals (experimental versus control: $P < 0.002$; control versus normal: $P > 0.05$).

Nerve terminals in experimental and control muscles were examined in the electron microscope to search for morphological correlates of this enhanced release. After processing the tissue as described previously⁴, micrographs of randomly chosen transverse sections ($\times 43,500$) were examined for differences in: (1) the number of vesicles in a standard 0.528- μm^2 area just above the presynaptic membrane, (2) the number of mitochondria, (3) cross-sectional area, and (4) the width and character of the synaptic cleft. As shown in Table 2, neither vesicle density

Table 2 Aspects of nerve terminal structure at experimental (re-innervated half sartorius) and control (re-innervated whole sartorius) junctions

	Experimental	Control	P
Vesicle density (no. vesicles per 0.528 μm^2)	7.5 $\pm$ 0.5 (55)	7.0 $\pm$ 0.4 (34)	> 0.05
No. mitochondria	3.0 $\pm$ 0.4 (65)	2.6 $\pm$ 0.4 (38)	> 0.05
Cross-sectional area (μm^2)	0.80 $\pm$ 0.06 (71)	1.10 $\pm$ 0.12 (38)	< 0.05
% Junctions with interposed Schwann cell processes	18% (17/95)	36% (15/42)	
% Junctions with unobstructed synaptic apposition	82% (78/95)	64% (27/42)	

Data shown are mean $\pm$ s.e.m., with number of sections in parentheses.

(possibly proportional to the number of readily releasable transmitter packets), nor the number of mitochondria (whose Ca^{2+} -sequestering activity may affect release¹⁰) differed significantly for experimental and control terminals. The cross-sectional area of control terminals did exceed that of experimental terminals by a marginally significant amount. We lack evidence for determining how such a difference could affect release. More relevant perhaps is our preliminary finding that control junctions show a higher incidence of interposition of Schwann cell processes between the pre- and post-synaptic membranes. Further observations on this point are in progress.

It seems, therefore, that the process of transmitter release at frog neuromuscular junctions can show plasticity, with the size of the peripheral field of a motoneurone an important determining factor. If such variability in motor unit size occurs normally, it could help explain the relatively poor correlation that has been found between quantal content and nerve terminal size^{4,6}. The dependence of release on motor unit size may, on the other hand, occur only as an experimental result in response to altered motor unit size. Further, it is not known if the increase in transmitter output per unit length is a permanent change.

Our data are consistent with the hypothesis that the level of transmitter output from a terminal may be related to the amount of material that that terminal receives from its soma. Production and/or transport of this material is fixed during development so that the supply is appropriate for the peripheral field of each motoneurone. If the field size is changed, the motoneurone may

Table 1 Quantal content at sartorius junctions in Ringer containing 0.3 mM Ca^{2+} and 1 mM Mg^{2+}

	Quantal content	Quanta released per 100 μm
Re-innervated half sartorius	6.30 $\pm$ 0.84 (34)	1.89 $\pm$ 0.43 (21)
Re-innervated whole sartorius	2.79 $\pm$ 0.59 (19)	0.46 $\pm$ 0.09 (16)
Normal sartorius	2.07 $\pm$ 0.36 (25)	0.45 $\pm$ 0.09 (24)

Data shown are mean $\pm$ s.e.m. (n). For re-innervated muscles, the sartorius nerve was crushed 59–84 days previously.

not compensate and one would expect to see a change in transmitter release. In the present experiments, for example, the original amount of this material would be distributed to a smaller number of terminals, resulting in enhanced transmitter release.

Further experiments are in progress to determine if an increase in the number of terminals per axon results in a decrease in transmitter release.

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Benzodiazepine recognition sites on GABA receptors

Moshe Gavish & Solomon H. Snyder*

Departments of Neuroscience, Pharmacology and Experimental Therapeutics, and Psychiatry and Behavioral Sciences, Johns Hopkins University School of Medicine, Baltimore, Maryland 21205

A role of γ -amino butyric acid (GABA) in benzodiazepine actions^{1–3} is supported by the GABA stimulation of membrane-bound^{4–8} and soluble^{9,10} benzodiazepine receptors and preliminary evidence that GABA protects benzodiazepine receptors from heat inactivation¹¹. We show here that the GABA receptors labelled by ³H-muscimol possess a benzodiazepine recognition site, as benzodiazepines, in proportion to their pharmacological activity, protect ³H-muscimol binding from inactivation by heat and the protein modifying reagent iodoacetamide. We also show that benzodiazepines and GABA act at apparently distinct sites to protect benzodiazepine receptors from heat and iodoacetamide.

Brain membranes were incubated with receptor 'protector' compounds, then were either heated or treated with iodoacetamide, and washed 15 times to remove the 'protectors'. In control membranes, seven washes restored ³H-muscimol binding to control values after addition of GABA, while 15 washes were required to restore ³H-flunitrazepam binding to control values after incubation with diazepam. The 15 routine washes used did not lower control binding of ³H-flunitrazepam or ³H-muscimol, presumably because any labile binding sites had already been degraded during the initial six washes used to remove endogenous GABA (Fig. 1).

GABA protects both ³H-flunitrazepam and ³H-muscimol binding from inactivation by heat or iodoacetamide (Fig. 1), but seems to be more effective in protecting ³H-muscimol binding. Addition of 10 μ M and 0.1 mM GABA apparently enhances ³H-muscimol binding after heating. This may be due to the removal of factors¹² tightly bound to receptors by heating, which resembles the increased binding with freezing and thawing or with detergents¹³. Preliminary experiments with briefer periods of heating produced the same enhanced ³H-muscimol binding in GABA-protected membranes.

The protection of flunitrazepam and muscimol binding by GABA reflects GABAergic synaptic actions, for diaminobutyric acid, structurally similar to GABA but devoid of GABAergic activity, fails to protect either ³H-flunitrazepam or ³H-muscimol binding from heat or iodoacetamide. In contrast,

imidazoleacetic acid (IAA), which is slightly weaker than GABA in its synaptic effects, is somewhat less potent than GABA in protecting ³H-flunitrazepam and ³H-muscimol binding from heat inactivation.

4, 5, 6, 7-Tetrahydroisoxazolo (5, 4-c) pyridine-3-ol (THIP) competes strongly for ³H-muscimol binding but does not stimulate benzodiazepine receptor binding¹⁴; it acts as an antagonist at the GABA recognition site on benzodiazepine receptors to block GABA stimulation of ³H-diazepam binding^{15,16}. The finding that both THIP and the GABA antagonist bicuculline protect ³H-flunitrazepam and ³H-muscimol binding sites from heat inactivation indicate that occupancy by either agonist or antagonist can protect the receptor.

Benzodiazepines also protect both ³H-flunitrazepam and ³H-muscimol sites from heat and iodoacetamide. This effect reflects an affinity for benzodiazepine receptors, because Ro 5-6227, a benzodiazepine with a similar receptor affinity to diazepam, protects as well as does diazepam, whereas RO5-4964 and halazepam, benzodiazepines with low, weak benzodiazepine receptor affinity, do not protect ³H-flunitrazepam and ³H-muscimol binding.

In saturation analyses using 6–8 concentrations of ³H-ligand, heat inactivation reduces numbers of binding sites (B_{max} in control and heated conditions, respectively, in fmol per mg protein are 440 and 142 for ³H-muscimol and 600 and 190 for ³H-flunitrazepam) for GABA and benzodiazepine receptors but has no effect on the dissociation constants (K_D : ³H-muscimol 10.1 nM, ³H-flunitrazepam 4.4 nM). Protection by GABA and diazepam involves changes in B_{max} values with no change in K_D . The increased values following heating observed for GABA-protected ³H-muscimol binding also reflect increased B_{max} with unaltered K_D .

To explore the specificity of these protective effects we evaluated whether diazepam can protect dopamine or muscarinic cholinergic receptors from heat inactivation. Heating membranes at 55 °C for 30 min reduces ³H-quinuclidinyl benzilate (³H-QNB) binding to calf whole brain membranes (muscarinic receptors) and ³H-spiroperidol binding to rat corpus striatum (dopamine receptors) by 90%. Pretreatment with 10 μ M diazepam in the same conditions that protected GABA and benzodiazepine receptors fails to protect dopamine and muscarinic receptor binding from heat inactivation. As an additional indication of specificity, chlorpromazine, haloperidol and phenothiazine pretreatment do not protect ³H-muscimol or ³H-flunitrazepam binding from heating.

To determine whether heat inactivation has similar effects on distinct GABA and benzodiazepine recognition sites on benzodiazepine receptors and whether protection by GABAergic and benzodiazepine compounds is exerted at both sites, we evaluated the influence of GABA (100 μ M) on

Table 1 Interactions of GABA analogues and benzodiazepines in protecting ³H-flunitrazepam and ³H-muscimol binding sites from heat inactivation

Drug concentration (μ M)	Per cent of unheated control	
	³ H-Flunitrazepam	³ H-Muscimol
GABA (10)	15 $\pm$ 2	17 $\pm$ 1
Diazepam (10)	76 $\pm$ 8	128 $\pm$ 12
Clonazepam (100)	48 $\pm$ 5	35 $\pm$ 3
IAA (100)	45 $\pm$ 4	32 $\pm$ 3
Bicuculline (100)	47 $\pm$ 6	80 $\pm$ 7
THIP (4,5,6,7-tetrahydroisoxazolo (5,4-c)pyridine-3-ol) (100)	49 $\pm$ 5	47 $\pm$ 5
GABA (10) + diazepam (10)	38 $\pm$ 4	98 $\pm$ 8
GABA (10) + clonazepam (10)	108 $\pm$ 9	194 $\pm$ 15
GABA (10) + IAA (100)	105 $\pm$ 7	188 $\pm$ 14
GABA (10) + bicuculline (100)	75 $\pm$ 6	130 $\pm$ 10
GABA (10) + THIP (100)	74 $\pm$ 5	135 $\pm$ 9
Diazepam (10) + IAA (100)	60 $\pm$ 4	109 $\pm$ 8
Diazepam (10) + bicuculline (100)	93 $\pm$ 8	140 $\pm$ 13
Diazepam (10) + THIP (100)	95 $\pm$ 8	79 $\pm$ 6
	98 $\pm$ 8	140 $\pm$ 11

Methods were essentially as described in Fig. 1. Combinations of protecting substances were added simultaneously. Data are means $\pm$ s.e.m. for 8–10 determinations.

* To whom correspondence should be addressed.

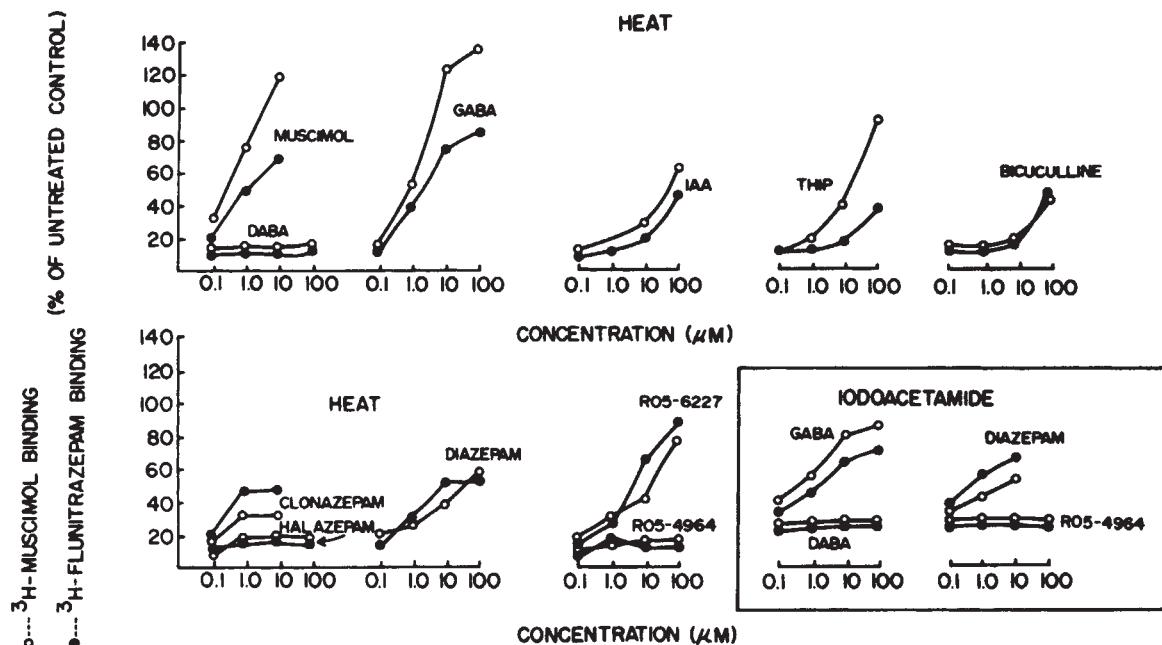


Fig. 1 Fresh calf brains were obtained from a slaughterhouse on ice within 30 min of death. Brains minus cerebella were homogenized with a Waring blender in 10 vol ice-cold 0.32 M sucrose and centrifuged 49,000g for 15 min at 4 °C. Pellets were resuspended in 2 vol 0.32 M sucrose and frozen for 18 h. After thawing, the tissue was washed five times by resuspension and recentrifugation with 40 vol 0.05 M Tris-HCl buffer (pH 7.4 at 4 °C) and refrozen for 18 h. After thawing, the tissue was washed once again by resuspension and recentrifugation with 40 vol 0.05 M Tris-HCl buffer. The pellet was then resuspended (70 μ g protein ml⁻¹) in Tris-HCl buffer. The tissue was incubated for 30 min at 0 °C with different concentrations of drugs followed by either heat inactivation (30 min at 55 °C) or iodoacetamide treatment (0.1 M iodoacetamide 30 min at 37 °C). Samples were washed 14 times with Tris-HCl buffer and once again with 0.05 M Tris-citrate buffer (pH 7.1 at 4 °C). Incubation media contained 1.7 ml tissue (120 μ g protein in Tris-citrate buffer), 0.1 ml ³H-flunitrazepam (final concentration 0.4 nM), or ³H-muscimol (final concentration 5.5 nM) and 0.2 ml of unlabelled drug (nonspecific binding) or H₂O (for total binding). To determine nonspecific binding incubations included 10 μ M clonazepam (for flunitrazepam binding) or 100 μ M GABA (for muscimol binding). After incubation (60 min at 0 °C) samples were filtered over GF/B filters and washed twice with cold Tris-HCl buffer. Filters were placed in vials containing 10 ml Formula 947 (NEN), shaken for 60 min and radioactivity was measured by liquid scintillation spectrometry. Data are means of 8–10 determinations. Protection from heating of both ³H-flunitrazepam and ³H-muscimol sites was significant ($P < 0.01$) for concentrations equal to or greater than 0.1 μ M muscimol, 1 μ M GABA, 10 μ M IAA 10–100 μ M THIP, 100 μ M bicuculline, 1 μ M clonazepam, 1 μ M diazepam, 1 μ M Ro 5-6227. Protection from iodoacetamide for ³H-muscimol and ³H-flunitrazepam was significant ($P < 0.01$) for concentrations equal to or exceeding 0.1 μ M GABA and 0.1 μ M diazepam. DABA, D,L-2,4-diaminobutyric acid.

³H-flunitrazepam binding. In control unheated membranes as well as in heated membranes this concentration of GABA enhances ³H-flunitrazepam binding about 60%. Moreover, half-maximal enhancement of ³H-flunitrazepam binding occurs at 0.9 μ M in both control and heated membranes. Additionally, in membranes treated with all the various protecting agents used in the present study, GABA (100 μ M) uniformly enhances ³H-flunitrazepam binding by 60%. Thus, GABAergic and benzodiazepine compounds protect the GABA recognition site of benzodiazepine receptors as well as their benzodiazepine recognition site.

Although GABA receptors clearly possess benzodiazepine recognition sites, we have failed to find stimulation or inhibition of ³H-muscimol binding with 10 nM–10 μ M clonazepam, an extremely potent benzodiazepine, and diazepam using Tris-citrate as well as Tris-HCl buffers.

To determine whether benzodiazepines and GABA protect the two receptors at the same or at different sites, we ascertained whether the effects of these substances were additive in protection experiments (Table 1). Maximal protection by GABA and IAA of ³H-flunitrazepam and ³H-muscimol binding is not additive, indicating that these substances act at the same sites. Might bicuculline or THIP antagonize GABA-elicited protection? Maximal protection of both ³H-flunitrazepam and ³H-muscimol sites by GABA and bicuculline is the same as with GABA alone, indicating no such antagonism. Protection by THIP and GABA for ³H-flunitrazepam and ³H-muscimol sites is slightly less than the maximal protection by GABA alone. In contrast to results with combinations of GABAergic substances, maximal effects of GABA and diazepam are even greater than the sum of the individual effects of these two compounds. Similarly, maximal effects of THIP and diazepam or IAA and diazepam are greater than the sum of the two substances acting separately. Thus, GABAergic agents and benzodiazepines exert their protective

effects at distinct recognition sites both on the receptors labelled by ³H-flunitrazepam and on those labelled by ³H-muscimol. The synergistic interactions of GABA and diazepam in protecting these receptors suggest that the two sites are allosterically linked.

Our major finding here is that GABA receptors labelled by ³H-muscimol possess recognition sites for benzodiazepines. Moreover, these sites have the same as or very similar drug specificity to benzodiazepine receptors. The findings that GABA receptors have benzodiazepine recognition sites and that benzodiazepine receptors have GABA recognition sites together with few differences in the chromatographic behaviour of the two purified receptors (M.G. and S. H. S., in preparation) indicates considerable structural similarity of the two receptor proteins.

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Uninfected avian cells contain structurally unrelated progenitors of viral sarcoma genes

Mitsuaki Yoshida*, Sadaaki Kawai†
& Kumao Toyoshima†

* Department of Viral Oncology, Cancer Institute, Toshima-ku, Tokyo, Japan

† Department of Oncology, Institute of Medical Science, University of Tokyo, Minato-ku, Tokyo, Japan

A single gene, *src* of Rous sarcoma virus (RSV) coding for a protein of pp60^{src}, is responsible for the transformation of fibroblasts¹⁻³. DNA sequences homologous to *src* (the endogenous *sarc*) are present in uninfected cells of chickens⁴ and other vertebrates⁵. Endogenous nucleotide sequences have also been found in the putative transforming genes of MC29 (ref. 6) and avian erythroblastosis virus⁷. These viruses, however, induce different spectra of tumours in animals. From analysis of a new avian sarcoma virus, Y73, we present here evidence suggesting that multiforms of viral sarcoma genes originated from cellular genetic sequences, and that these viral genes are structurally unrelated, but have similar pathogenicities.

Y73, a strain of avian sarcoma virus newly isolated from a spontaneous tumour of White Leghorn⁸, is a defective virus inducing fibrosarcoma in chickens but not any type of acute leukaemia, and contains 26S RNA as its genome⁹. To characterize the transforming gene (*yas*) of Y73, we prepared cDNA specific to 26S genome RNA (cDNA_{yas}) by transcribing 26S RNA into cDNA and selecting single-stranded cDNA after hybridization with a large excess of 35S genome RNA of Y73-associated virus (YAV). The specificity of the ³²P-cDNA_{yas} obtained was confirmed by the fact that the preparation showed no detectable hybridization with RNA of YAV or with 28S ribosomal RNA, which might contaminate the 26S RNA fraction owing to its similar size. This specific ³²P-cDNA_{yas} would represent about 2.5 kilobases of the middle portion of 26S RNA genome, because fingerprinting analysis of 26S RNA showed that 1–1.5 kilobases of both ends are common to 35S RNA of YAV leaving about 2.5 kilobases in the middle as specific sequence (manuscript in preparation).

The ³²P-cDNA_{yas} was hybridized with RNAs of various sarcoma and acute leukosis viruses in stringent conditions (Table 1). Most of the ³²P-cDNA_{yas} hybridized with 50–70S or 26S RNA of Y73, but not with the other virus RNAs tested (that is RNAs of avian myeloblastosis virus, avian erythroblastosis virus, MC29, the Prague strain of RSV, Fujinami sarcoma virus (FSV)¹⁰ and PRCII sarcoma virus¹¹). Hybridization of ³²P-cDNA_{total} indicated the presence of an appreciable amount of viral RNA and less than 1% of the viral RNA could easily be detected in the conditions used. These results clearly indicate that the Y73-specific sequences have no homology with the transforming sequences of known avian retroviruses. In particular, it should be emphasized that cDNA_{yas} is not structurally related to *src* of RSV or the transforming gene of FSV or PRCII. We also showed that cDNA_{src} of RSV did not cross-hybridize with the RNAs of Y73, FSV and PRCII (Table 1). Lee *et al.*¹² and Hanafusa *et al.*¹³ recently reported that there is no sequence homology between *src* of RSV and FSV. These findings show that Y73 is a new type of avian sarcoma virus and that avian sarcoma viruses contain structurally distinct multiforms of sarcoma genes. The putative transforming genes of MC29 (ref. 6) and AEV¹⁴, which can transform chicken fibroblasts *in vitro*, were reported to be distinct from *src* of RSV, but these viruses have different target specificity in animals. Our findings demonstrate that at least three structurally unrelated genes can induce similar fibrosarcomas in chickens.

As the *src* sequences of RSV were shown to be derived from host cell sequences^{4,15}, it was interesting to test whether the

sarcoma sequences in Y73, which are unrelated to *src* of RSV, also originated from the avian genome. Thus, we analysed restriction fragments hybridizable with cDNA_{yas} by the blotting procedure of Southern¹⁶. DNA of uninfected chicken cells was digested with various restriction endonucleases and the fragments separated by electrophoresis in 0.8% agarose and fixed on a nitrocellulose filter. When the filter was hybridized with total ³²P-cDNA_{RSV}, many bands of the endogenous viruses, as well as those of the endogenous *sarc*, were detected in digestions with *Eco*RI, *Hind*III, *Bgl*II and *Kpn*I (Fig. 1, *a* lanes). With ³²P-cDNA_{src} prepared from RSV, only a few bands representing endogenous *sarc* were detected in each digest (Fig. 1, *b* lanes). For example, in the digest with *Hind*III, two bands of approximately 12 and 28 kilobases were detected with ³²P-cDNA_{src} (lane *b*) and these bands corresponded to two of five bands detected with ³²P-cDNA_{RSV} (lane *a*). The fact that the three other bands could not be detected with cDNA_{src} showed that there was no significant contamination of the probe with other portions of the viral gene and thus that the fragments of 12 and 28 kilobases are those of the endogenous *sarc* sequences. Similar results were obtained with other enzyme digests, thus confirming the reliability of the method used.

When ³²P-cDNA_{yas} was used to hybridize the filter, only a few bands were detected in each digest (Fig. 1, *c* lanes) and the bands did not correspond to those of endogenous *sarc* in *b* lanes or those of endogenous viral gene in *a* lanes. These results unequivocally demonstrate that chicken DNA contains nucleotide sequences related to transforming gene of Y73 and that these endogenous DNA sequences (c-*yas*) are different from the endogenous *sarc* related to *src* of RSV and do not correspond to any endogenous viral sequences. Furthermore, the intensities of

Table 1 Absence of sequence homology of Y73-specific sequences with other avian sarcoma-leukosis viruses

		³² P-cDNA (% hybridized)		
RNA		cDNA _{yas}	cDNA _{src}	cDNA _{total}
Sarcoma viruses				
Y73	50–70S	91	4	98
Y73	26S	89	2	95
Y73-associated virus	35S	2	2	85
RSV(Pr-RSV-C)	39S	1	96	52
Y73	Cellular*	78	–	–
Fujinami virus	Cellular*	11	10	65
PRCII virus	Cellular*	10	9	53
Noninfected	Cellular*	9	5	6
Acute leukosis viruses				
AEV	30S	1	–	45
AMV	50–70S	3	–	65
MC29	Q8†			
	Cellular	11	–	21

Hybridization was carried out at 42 °C in buffer containing 0.6M NaCl, 50 mM Tris-HCl (pH 7.5), 1 mM EDTA and 50% formamide to a *C_tt* value of 15 mol s l⁻¹ with purified virion RNAs or 600 mol s l⁻¹ with total cytoplasmic RNA. The *C_tt* values (600 mol s l⁻¹) for cellular RNA were 50–70-fold in excess of *C_tt* for hybridization between cDNA_{total} and individual RNA preparation. ³²P-cDNA_{yas} and ³²P-cDNA_{total} were prepared as follows: the 26S RNA fraction of Y73 was transcribed into ³²P-cDNA_{total} with AMV reverse transcriptase in the presence of [α-³²P]dCTP and random primer of calf thymus DNA digested with DNase I as described by Taylor *et al.*¹⁷. The ³²P-cDNA_{total} was hybridized with a large excess of 35S RNA of Y73-associated virus (YAV) to a *C_tt* of 100–150 mol s l⁻¹ and single-stranded DNA, ³²P-cDNA_{yas}, was separated on a hydroxyapatite column. ³²P-cDNA_{src} of RSV was prepared as described by Stehelin *et al.*¹⁸ from the Prague strain of RSV and RNA of the transformation-defective mutant of the strain (td-RSV).

* Total cytoplasmic RNA was extracted from cells infected and fully transformed with the viruses and the contents of defective viral RNA were estimated to be more than 20% of the helper 35S RNA by the Northern transfer technique¹⁹; thus, if they have any homology, significant hybridization should be observed in the conditions used.

† Q8 is a nonproducer quail cell line²⁰.

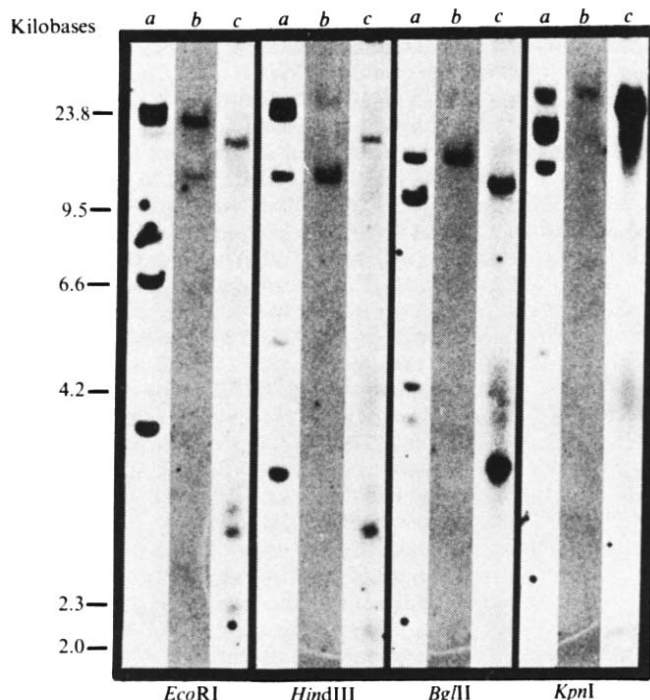


Fig. 1 Detection of nucleotide sequences homologous to the putative transforming gene of Y73 in uninfected chicken DNA and their comparison with endogenous *sarc* sequences related to *src* of RSV. DNA was extracted from uninfected chicken embryo fibroblasts and digested with *Eco*RI, *Hind*III, *Bgl*II and *Kpn*I respectively. Each digest was introduced into three slots in 0.8% agarose. The DNA fragments were separated by electrophoresis and blotted on to a nitrocellulose filter as described by Southern¹². The three strips of the filter of each digestion were separately hybridized with total ³²P-cDNA_{RSV} (a) ³²P-cDNA_{Y73} that had been prehybridized with RNA of td-RSV (b), and ³²P-cDNA_{YAV} that had been prehybridized with RNA of YAV (c), and autoradiograms were re-aligned. The marker for molecular size was λ DNA digested with *Hind*III.

the bands detected using ³²P-cDNA_{Y73} suggested that one or a few copies of *c-yas* sequences are present in chicken DNA. These findings strongly suggest that Y73 acquired the transforming gene from the *c-yas* sequences and that it belongs to a distinct class of avian sarcoma viruses. In preliminary results, the *c-yas* sequences are transcribed into RNA containing poly (A), suggesting that this RNA is translated into protein(s).

Our findings on the probable cellular origin of the putative transforming gene (*yas*) of Y73 indicate that the avian genome contains at least two copies of structurally unrelated progenitors of sarcoma genes of avian sarcoma viruses. These findings suggest that spontaneous or chemically induced sarcomagenesis might involve some modification and/or abnormally activated expression of one of these progenitor sequences, including as yet unidentified sequences. This hypothesis could be tested using a set of cDNAs specific to various classes of sarcoma genes.

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The *Agrobacterium tumefaciens* Ti plasmid as a host vector system for introducing foreign DNA in plant cells

Jean-Pierre Hernalsteens

Laboratorium voor Genetische Virologie, Vrije Universiteit Brussel, B-1640 Sint-Genesius-Rode, Belgium

Françoise Van Vliet, Marc De Beuckeleer, Ann Depicker, Gilbert Engler, Michel Lemmers, Marcelle Holsters, Marc Van Montagu & Jeff Schell*

Laboratoria voor Histologie en Algemene Genetika, Rijksuniversiteit Gent, B-9000 Gent, Belgium

The molecular basis of the neoplastic transformation of plant cells by the crown gall bacterium *Agrobacterium tumefaciens* is the transfer to and stable maintenance of T DNA—a well defined segment of the bacterial Ti plasmid—in plant cells¹⁻⁴. Transformed cells express new biosynthetic capacities, such as the synthesis of opines⁵, of which octopine⁶ and nopaline⁷ are well known examples. Ti plasmids harbour genes enabling the bacteria to degrade opines and use them as carbon and nitrogen sources⁸. pTi-linked genes also determine the specificity of synthesis of opines in the plant tumour cells⁹. On this basis the Ti plasmid can be considered an unusual type of catabolic plasmid, which induces the synthesis of its substrate in transformed plant cells. This relationship, called genetic colonization², has opened prospects for the use of Ti plasmids as vectors for genetic manipulation of plants. To evaluate this possibility we inserted a well defined DNA segment, the bacterial transposon Tn7 (ref. 9), into the Ti-plasmid DNA sequence that determines nopaline synthesis in *A. tumefaciens* strain T37 Noc¹ (ref. 10). As we report here, the inserted Tn7 DNA segment became part of the T DNA because the 9.6×10^6 molecular weight (MW) Tn7 DNA sequence was transferred to, and maintained in, the DNA of tumour tissue cultures induced by this mutant strain.

Tn7, which encodes streptomycin, spectinomycin and trimethoprim resistance, was introduced into the chromosome of strain T37 Noc¹. An RP4::Tn7 plasmid⁹, pGV1 (our isolate) was transferred from the auxotrophic *Escherichia coli* strain GV1000 (ref. 11) to strain T37 Noc¹ by plate conjugation¹². A transconjugant subsequently cured of its pGV1 plasmid was obtained by selection with the pilus-specific bacteriophage GU5 (J. P. H., in preparation). A culture of a transconjugant colony, exponentially growing in LB medium, was infected with a multiplicity of 5 plaque-forming units of GU5 per bacterium and plated after overnight incubation at 32 °C. About 15% of surviving cells were shown to have lost the carbenicillin, kanamycin and tetracycline resistance markers of RP4 but retained the spectinomycin (100 μ g ml⁻¹) and trimethoprim (1,000 μ g ml⁻¹) resistance markers of the Tn7 transposon.

One such T37 Noc¹ chr::Tn7 strain, GV15, was used for the isolation of Tn7 insertions in the transfer-derepressed Ti plasmid of strain T37 Noc¹ by selecting for the Ti-plasmid mediated conjugative transfer of the transposon resistance markers.

* Max Planck Institut für Züchtungsforschung, 30 Köln 5000, FRG.

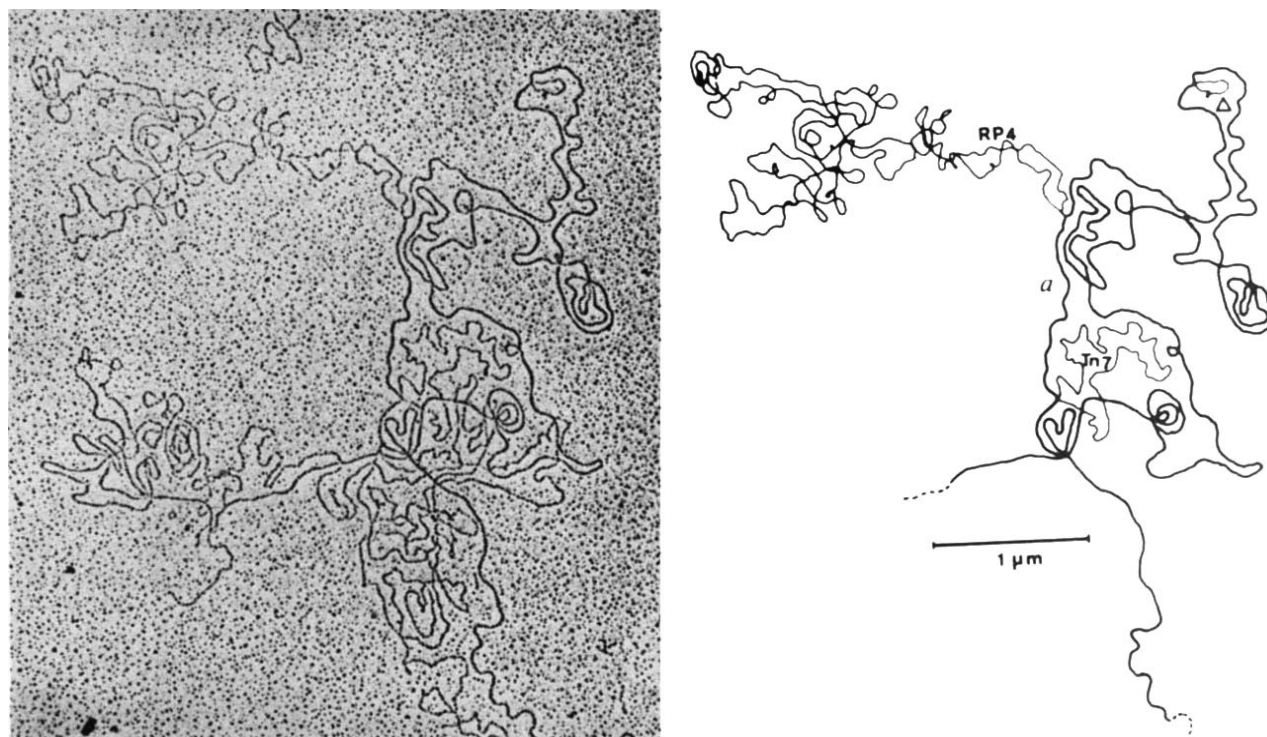
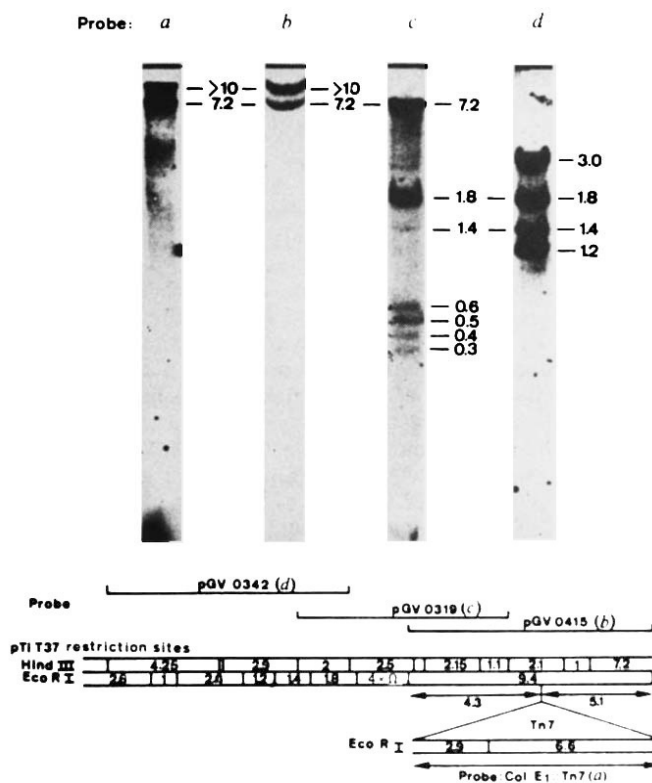


Fig. 1 Physical mapping of the Tn7 insertion in a nopaline-synthesis deficient (Nos⁻) mutant of pTiT37. pGV3106 DNA was prepared from strain GV3171 as described previously¹². The Tn7 insertion in pGV3106 was localized by electron microscopic analysis of heteroduplex molecules²⁰ formed by renaturation of single-stranded DNA molecules of pGV3106 and of pGV4000 (ref. 18), a co-integrate plasmid consisting of pTiC58 and the P plasmid RP4. The distance (*a*) between the Tn7 transposon in pGV3106 and the RP4 insertion (at map position 130.4)¹⁸ in pGV4000 is $1.3 \pm 0.1 \mu\text{m}$. The 0.5- μm deletion loop (Δ) in pTiT37 (ref. 4) and present in pGV3106 (at map position 124.3–125.3) (G. E., in preparation) was used as a reference point for the Tn7 integration site. This mapping result agrees with the restriction endonuclease analysis (data not shown) of pGV3106.

Fig. 2 Analysis of Tn7 and Ti-plasmid related DNA fragments present in restriction endonuclease digests of tobacco tumour DNA. W38T37::Tn7-1, a bacterium-free tumour tissue culture, was started from a tumour induced by strain GV3171 on *N. tabacum* cv Wisconsin 38, essentially as described previously¹⁶. After 5 months of *in vitro* culture on hormone-free medium, samples of the tissue were extracted with 25 ml buffer (Tris 50mM pH 8.2, EDTA 100 mM, diethylammonium diethyl-dithiocarbamate (DIECA) 100mM, triisopropyl naphthalene sulphonic acid 2%, diethylpyrocarbonate 0.1%) per g lyophilized tissue. The mixture was brought to 0.3 M NaCl and extracted with an equal volume of phenol and chloroform. The aqueous phase was brought to 0.5 M NaCl and re-extracted with the same organic phase. After precipitation with two volumes of ethanol the crude DNA was further purified by CsCl gradient banding (starting density 1.55). After dialysis it was concentrated to about 1 mg ml^{-1} by ethanol precipitation. The DNA was digested to completion with restriction endonuclease *Eco*RI. Digested DNA (20 μg) was separated by electrophoresis through a 0.8% agarose slab gel and transferred to a nitrocellulose filter¹⁹. The filter was hybridized by Southern's¹⁹ method to cloned Ti-plasmid fragments derived from the T region of pTiC58 (ref. 17) and to ColE1::Tn7 plasmid DNA, labelled *in vitro* with ³²P by nick-translation²¹. ColE1::Tn7 plasmid DNA was used as a probe in lane *a*. The extent of the cloned pTi fragments used as probes for the hybridizations of lanes *b*, *c* and *d* is indicated on top of the physical map of the T region of pTiT37. Lanes *a* and *b* demonstrate the presence in the tumour DNA of the complete Tn7 sequence integrated in a sequence homologous to the 9.4×10^6 MW *Eco*RI fragment of pTiT37. The localization of the integration site of Tn7 in the T-DNA segment based on these data and confirmed by similar hybridization experiments with *Hind*III digests of the tumour DNA (data not shown), corresponds to the integration site of Tn7 in pGV3106 (Fig. 1). Lanes *b*, *c* and *d* demonstrate that the T-DNA segment in this tumour line is identical, except for the *Eco*RI fragment of MW 9.4×10^6 , to the T-DNA segment independently shown to be present in the BT37 tobacco tumour line induced by wild-type pTiT37 (ref. 4). Numbers adjacent to bands and numbers in each segment indicate MW ($\times 10^{-6}$).



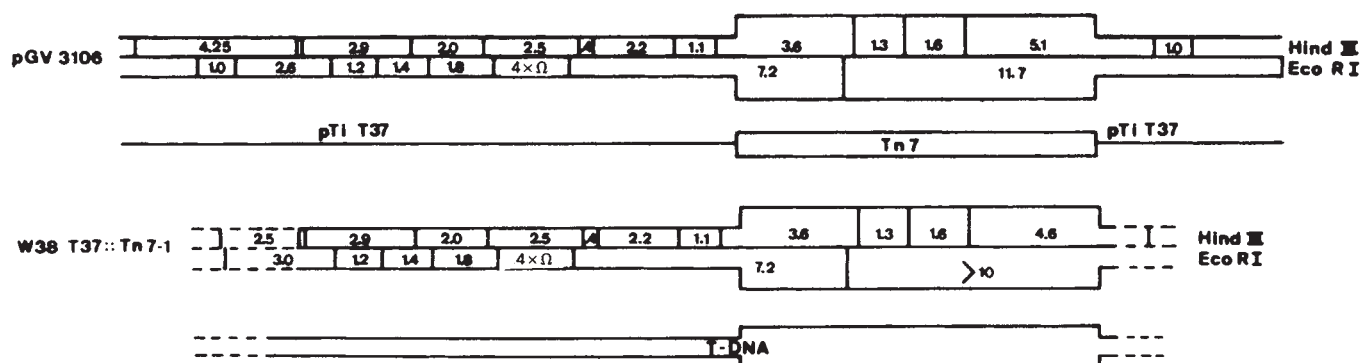


Fig. 3 The colinearity between the T region of the pTiT37::Tn7 plasmid pGV3106 and the T-DNA segment in the tumour line W38T37::Tn7-1 induced by the *A. tumefaciens* strain GV3171 harbouring pGV3106. The figure is based on results obtained by Southern type DNA hybridizations as described in Fig. 2 legend.

GV15 was incubated for 24 h on minimal A¹³ agar with the rifampicin- and erythromycin-resistant C58C1 derivative GV3102. Tn7-containing transconjugants were found at a frequency of 1.5×10^{-5} per recipient cell after plating on LB¹³ medium supplemented with rifampicin ($100 \mu\text{g ml}^{-1}$), erythromycin ($100 \mu\text{g ml}^{-1}$) and spectinomycin ($100 \mu\text{g ml}^{-1}$). In 10% of these strains Ti plasmid could be demonstrated by its ability to degrade nopaline⁸ and sensitivity to agrocin 84 (ref. 14). The remaining transconjugants expressed none of the Ti-plasmid markers and therefore probably harboured Tn7 insertions in the cryptic conjugative plasmids of strain T37Noc^{c1} (G. E., unpublished). Twelve transconjugants expressing both pTi and Tn7 markers were inoculated on sunflower hypocotyl segments¹⁵. Nine were found to be pathogenic. Nopaline synthesis was demonstrated reproducibly by electrophoretic analysis¹⁶ of the tumours induced by eight of these strains, but could not be detected in tumours induced by strain GV3171. The inability of strain GV3171 to induce nopaline synthesis was confirmed by analysis of tumours induced on potato, *Kalanchoë daigremontiana* and tobacco.

The Tn7 insertion in strain GV3171 was localized on the mutant Ti plasmid (pGV3106) by restriction endonuclease analysis, and mapped in a DNA fragment homologous to the HindIII-23 fragment¹⁷ of the standard nopaline Ti plasmid pTiC58 (data not shown). This localization was confirmed independently by electron microscopic heteroduplex analysis, which also showed that the Tn7 inserted without detectable deletion (Fig. 1). This HindIII-23 fragment was subsequently shown to be homologous with the right-end border fragment of the TDNA^{2,4} and thus to be part of the Ti-plasmid DNA segment which is normally transferred to plant cells. To confirm this result, several hundred Tn1 and Tn7 insertions in pTiC58 were screened for nopaline synthesis after induction of tumours. A single nopaline synthesis-deficient mutant was found and shown to be the result of a Tn1 insertion in fragment HindIII-23 (ref. 18).

A bacterium-free tissue culture, W38T37::Tn7-1, was started from a tumour induced by strain GV3171 on *Nicotiana tabacum* cv Wisconsin 38. Analysis by Southern blot hybridizations¹⁹ of a DNA sample isolated from this culture, using ColE1::Tn7 (our isolate) DNA and various cloned segments of the nopaline plasmid pTiC58 as radioactive probes showed that the complete Tn7 was present in the plant tumour genome, integrated at its original site in a normal-size T DNA (Figs. 2, 3).

Our results show that the Ti plasmid can be used as a vector for the experimental introduction and stable maintenance of foreign genes in higher plants. Furthermore, the demonstration that the Ti-plasmid DNA segment specifying nopaline synthesis in transformed plant cells is part of the TDNA, but not functionally required for the induction or maintenance of the neoplastic state, supports the genetic colonization model of crown gall².

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Transforming genes of neoplasms induced by avian lymphoid leukaemia viruses

Geoffrey M. Cooper* & Paul E. Neiman†

* Sidney Farber Cancer Institute and Department of Pathology, Harvard Medical School, Boston, Massachusetts 02115

† The Fred Hutchinson Cancer Research Center, Seattle, Washington 98104

Oncogenic avian retroviruses can be classified into three groups: sarcoma viruses, acute leukaemia viruses and lymphoid leukaemia viruses (LLVs)¹. Sarcoma and acute leukaemia viruses transform fibroblasts and/or haematopoietic cells in culture and induce tumours with short latent periods in infected birds. In contrast, LLVs do not transform cells *in vitro* and require long latent periods before formation of neoplasms *in vivo*. The most frequent neoplasm induced by LLVs is malignant lymphoma of the bursa of Fabricius, but LLVs also induce other neoplasms,

including sarcomas, nephroblastomas and erythroblastosis². The genomes of both sarcoma and acute leukaemia viruses contain specific genes responsible for viral oncogenicity^{1,3-5}, whereas the genome of LLVs apparently includes only genes required for virus replication. The genetic basis for the low oncogenic potential of LLVs is therefore obscure. The present experiments indicate that LLV-induced tumours contain transforming genes that can be detected by transfection of NIH 3T3 mouse cells. These transforming genes are not linked to LLV DNA sequences, suggesting that oncogenesis by LLVs may result from indirect activation of cellular transforming genes.

DNAs used as donors in transfection experiments were extracted from seven different LLV-induced chicken tumours, from LLV-infected non-neoplastic tissues of tumour-bearing birds and from tissues of uninfected birds. The tumours included a nephroblastoma, three metastatic bursal lymphomas, a cell line established from such a lymphoma and two neoplastic bursal nodules, which are the presumed precursors to metastatic lymphomas. LLV DNAs in the tumours and LLV-infected normal tissues used in these experiments have been characterized by restriction endonuclease analysis⁶. The bursal nodules and the nephroblastoma appeared to contain single complete exogenous LLV genomes integrated at a different site in each tumour⁶. The advanced metastatic lymphomas and the derivative cell line contained variable numbers of LLV DNA

integration sites, some of which corresponded to incomplete proviruses (ref. 6 and unpublished observations). In addition, recent studies using a probe homologous to the terminal sequences of LLV DNA (see below) revealed additional exogenous LLV DNA sequences in these tumour DNAs that were only detectable by this selective probe (unpublished observations). Red blood cells and normal bursal and kidney tissues from LLV-infected birds contained exogenous LLV genomes in quantities similar to LLV-induced tumours⁶.

Table 1 Transformation by DNAs of LLV-induced tumours

Donor DNA (tissue-bird no.)	Total colonies/ total recipient cultures	Colonies per μg DNA
Salmon sperm	0/36	<0.002
Uninfected:		
Bursa 2407	0/4	<0.01
Erythrocyte 2407	0/5	<0.01
Total	0/9	<0.005
LLV-infected normal:		
Bursa 2993	0/5	<0.01
2999	0/3	<0.02
B7655	0/8	<0.01
Erythrocyte 2902	0/7	<0.01
2993	0/4	<0.01
2999	0/10	<0.005
Kidney 3000	0/4	<0.01
Liver 2993	0/4	<0.01
2999	1/6	0.01
Total	1/43	0.001
LLV-induced tumours:		
Nodule 2993	5/4	0.06
2999	54/3	0.9
Metastatic 2902	2/6	0.02
lymphoma B7362	38/21	0.09
B7363	5/7	0.04
Lymphoma line LSCC-RP9	9/5	0.09
Nephroblastoma 3000	24/3	0.4
Total	137/49	0.14

DNAs were extracted from normal and neoplastic tissues of chickens infected with LLV strain 5938 (bird nos. 2902, 2993, 2999, 3000, B7655, B7362 and B7363), from normal bursa and erythrocytes of an uninfected chicken (no. 2407) of the same flock as chickens 2902, 2993, 2999 and 3000 and from an early (6th) tissue culture passage of a cell line (LSCC-RP9)²⁵ established from a transplantable bursal lymphoma (LSCC-RP6) induced by LLV strain Rous-associated virus-2. LLV infection of birds B7655, B7362 and B7363 was described by Neiman *et al.*²⁶ and LLV infection of birds 2902, 2993, 2999 and 3000 by Neiman *et al.*⁶. High molecular weight ($>20 \times 10^6$) DNAs were assayed by transfection of NIH 3T3 mouse cells as previously described¹³. Salmon sperm DNA was included as a control in all experiments. Recipient cultures were inoculated with 20 μg DNA per culture and were transferred into soft agarose-containing medium 7 d after exposure to DNA. Colonies of transformed cells were counted after 2-3 weeks of further incubation at 37 °C.

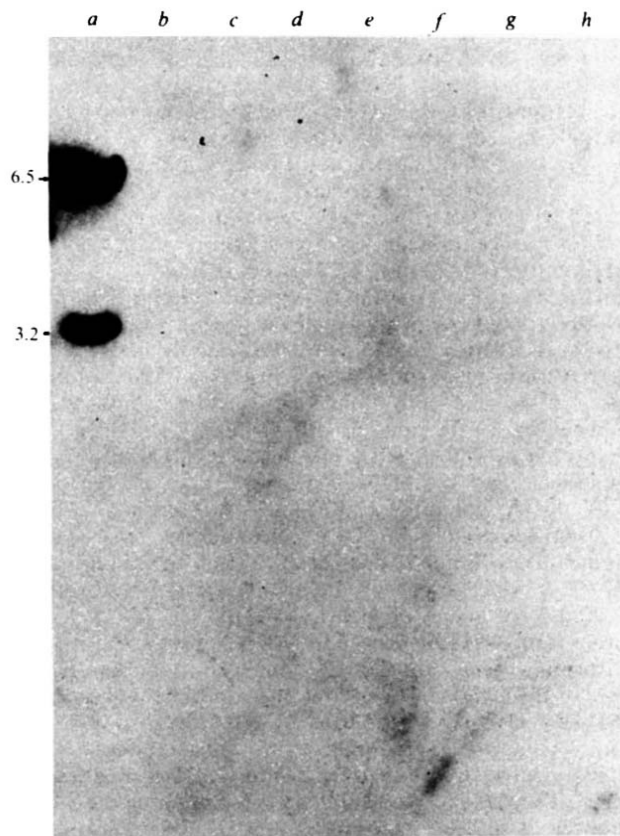


Fig. 1 Absence of LLV genomes in transformed NIH cells. DNAs of NIH cells transformed by Schmidt-Ruppin-D avian sarcoma virus DNA (lane a), NIH(BN-2993 DNA) cells (lane b), NIH(BN-2999 DNA) clone 1 cells (lane c), NIH(BN-2999 DNA) clone 2 cells (lane d), NIH(BN-2999 DNA) clone 3 cells (lane e), NIH(BL-B7362 DNA) cells (lane f), NIH(BL-LSCC-RP9 DNA) cells (lane g) and NIH 3T3 cells (lane h) were digested with *Hind*III, electrophoresed in 0.8% agarose gels and transferred to nitrocellulose filters as previously described⁹. Filters were baked and hybridized¹⁰ to ³²P-cDNA (specific activity, $\sim 4 \times 10^8$ cpm μg^{-1}) representative of the entire LLV genome which was synthesized by avian myeloblastosis virus reverse transcriptase from a template of Rous-associated virus-2 poly(A)-containing 35S RNA using oligomers of salmon sperm DNA as primers²⁷. Filters were washed and exposed to X-ray film as previously described¹⁰. The molecular weights ($\times 10^{-6}$) of LLV DNA-containing fragments are indicated.

The transforming activity of high molecular weight DNAs of tumours and normal tissues was assayed by transfection of NIH 3T3 cells, which have previously been shown to be transformable by DNAs corresponding to both viral and cellular transforming genes⁷⁻¹³. DNAs of all seven tumours, but not DNAs of either uninfected or LLV-infected normal tissues, induced transformation with efficiencies of 0.02-0.9 transformants per μg DNA (Table 1). These efficiencies were similar to the efficiencies of transformation by high molecular weight DNAs of cells transformed by avian sarcoma⁹ and acute leukaemia¹¹ viruses and by DNAs of cells containing activated cellular

transforming genes^{12,13}, but were 10–100-fold higher than the efficiency of transformation by fragments of normal cell DNA¹³. Thus, LLV-induced tumours, but not LLV-infected normal tissues, appeared to contain activated transforming genes that efficiently induced transformation of NIH 3T3 cells.

Several colonies of NIH cells transformed by LLV-induced tumour DNAs were grown to mass culture for further study. DNAs of these cells induced transformation in secondary transfection assays with similar efficiencies to the original efficiency of transformation by DNAs of LLV-induced tumours (~0.1 transformant per μg DNA) (Table 2) indicating they contained activated transforming genes. In contrast, transformation was not induced by DNAs of NIH 3T3 cells or spontaneously transformed NIH cells^{9,13} (Table 2).

The content of LLV DNA in NIH cells transformed by DNAs of LLV-induced tumours was investigated by restriction endonuclease analysis. DNAs of the transformed NIH cells used as DNA donors in the experiments presented in Table 2 were digested with *Hind*III, electrophoresed in agarose gels, transferred to nitrocellulose filters¹⁴ and hybridized to ³²P-cDNA representative of the entire LLV genome (Fig. 1). The sites of *Hind*III cleavage in viral DNAs are illustrated in Fig. 2. Two virus DNA fragments with molecular weights of 6.5 and 3.2×10^6 were readily detectable in *Hind*III-digested DNA of NIH cells transformed by avian sarcoma virus DNA and containing a single viral genome⁹ (Fig. 1, lane *a*). In contrast, no LLV DNA sequences were detectable in any of the six lines of NIH cells transformed by DNAs of LLV-induced tumours (Fig. 1, lanes *b–g*), indicating that these cells did not contain LLV genomes.

LLV DNA contains a 300-nucleotide terminal repeat, consisting of approximately 200 nucleotides derived from the 3' terminus and 100 nucleotides derived from the 5' terminus of LLV RNA, which appears to be involved in synthesis, integration and expression of proviral DNA^{15–18}. To determine whether terminal sequences of LLV DNA were associated with transforming genes of the LLV-induced tumours, *Hind*III digests of the same DNAs used in the experiment presented in Fig. 1 were also hybridized to ³²P-DNA of plasmid p53. This plasmid contains approximately 900 nucleotides of avian sarcoma virus DNA derived from *in vitro* DNA synthesis and cloned in the *Pst*I site of pBR322 and includes both the 3' and 5' sequences of the terminal repeat unit (K. Beemon, personal communications), and extends into the avian sarcoma virus *src* gene (unpublished observations, see Fig. 2). p53 ³²P-DNA hybridized to both *Hind*III fragments of DNA of avian sarcoma virus DNA transformed NIH cells (Fig. 2, lane *a*). Since the *Hind*III fragment of molecular weight 3.2×10^6 of this cell line is derived from the left-hand portion of avian sarcoma virus DNA and is homologous to p53 only at the terminal repeat unit (see

Fig. 2), hybridization to this fragment shows the sensitivity of detection of these sequences. p53 ³²P-DNA also hybridized weakly to *Hind*III fragments of molecular weights 5.5 and 2.9×10^6 d in DNAs of all transformed cell lines (Fig. 2, lanes *a–g*) and in DNA of normal NIH 3T3 cells (Fig. 2, lane *h*). These

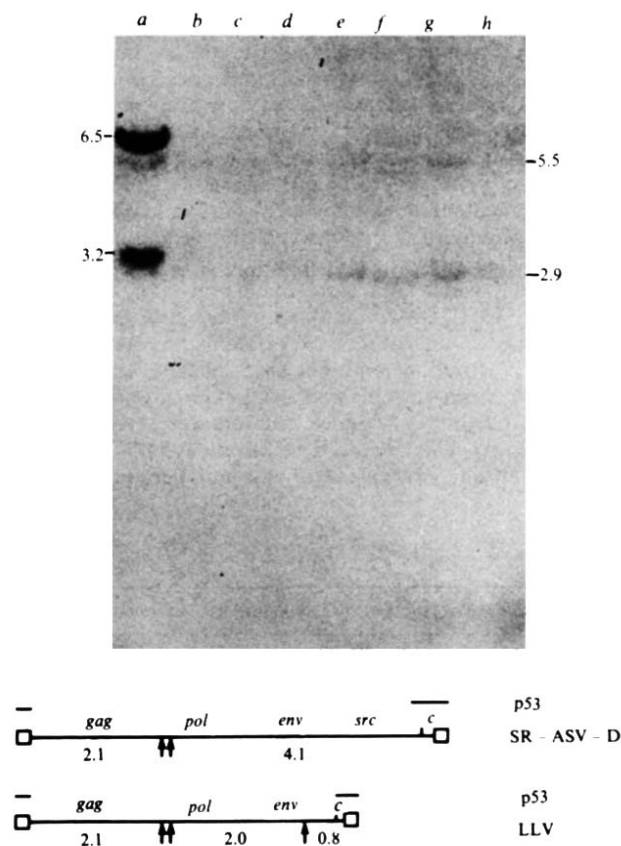


Fig. 2 Absence of terminal sequences of LLV DNA in transformed NIH cells. *Hind*III-digested DNAs of the same cell lines as were used in the experiment presented in Fig. 1 were electrophoresed, transferred to nitrocellulose filters and hybridized to ³²P-DNA synthesized by nick translation²⁸ of p53 (specific activity, $0.5\text{--}1 \times 10^8$ cpm μg^{-1}). Prehybridization and hybridization conditions were as described by Wahl *et al.*²⁹. The structure of Schmidt-Ruppin-D avian sarcoma virus (SR-ASV-D) and LLV unintegrated linear DNAs are shown below the autoradiogram. *Hind*III cleavage sites are indicated by arrows and the molecular weights ($\times 10^{-6}$) of *Hind*III fragments are given¹⁸. The *Hind*III site located in *env* is not present in SR-ASV-D DNA^{9,18} but may be present in some strains of LLV¹⁸. The terminal repeat unit of LLV DNA is shown as a box. The regions of viral DNA homologous to p53 are indicated above each map.

Table 2 Transformation by DNAs of transformed NIH cells

Donor DNA	Total colonies/ total recipient cultures	Colonies per μg DNA
Salmon sperm	0/28	<0.002
NIH 3T3	0/23	<0.002
NIH (spontaneous)	0/12	<0.005
NIH(BN-2993 DNA)	10/12	0.04
NIH(BN-2999 DNA)cl 1	23/18	0.06
NIH(BN-2999 DNA)cl 2	56/21	0.13
NIH(BN-2999 DNA)cl 3	48/16	0.15
NIH(BL-B7362 DNA)	17/15	0.06
NIH(BL-LSCC-RP9 DNA)	35/22	0.08

Salmon sperm DNA, NIH 3T3 cell DNA, DNA of spontaneously transformed NIH cells [NIH(spontaneous)] and DNAs of NIH cells transformed by DNAs of bursal nodule-2993 [NIH(BN-2993 DNA)], bursal nodule-2999 [NIH(BN-2999 DNA)clones 1, 2 and 3], bursal lymphoma-B7362 [NIH(BL-B7362 DNA)] and bursal lymphoma LSCC-RP9 [NIH(BL-LSCC-RP9) DNA] were assayed as described in Table 1 legend.

fragments may represent normal mouse sequences homologous to *src*¹⁹, since faint hybridization to these fragments was also detected with ³²P-cDNA representative of avian sarcoma virus RNA (unpublished observations). No additional LLV DNA sequences were present in NIH cells transformed by LLV-induced tumour DNAs (Fig. 2, lanes *b–g*), indicating that transforming genes of these cells were not associated with the terminal sequences of LLV DNA.

Thus, LLV-induced tumours contained cellular transforming genes, detectable by transfection of NIH 3T3 cells, which were not linked to LLV DNA. As transformation of NIH 3T3 cells was induced by DNAs of all seven tumours studied, including DNAs of two bursal nodules which represent an early stage of lymphoma development, it seems likely that activation of cellular transforming genes to a state in which they can be efficiently transmitted by transfection is significant in oncogenesis by

LLVs. That transforming genes of LLV-induced tumours are not linked to LLV DNA suggests that oncogenesis by LLVs is not a consequence either of expression of an LLV transforming gene or of formation of recombinant transforming viruses and, furthermore, does not support the hypothesis that transformation results from viral promoters acting on adjacent cellular genes²⁰. Instead, our results suggest that oncogenesis by LLVs may involve indirect activation of cellular transforming genes, possibly as a consequence of virus-induced mutations or gene rearrangements leading to abnormal gene expression. These events might be similar to those occurring in some chemically transformed mouse cell lines¹² or in the activation of potential transforming genes of normal cells during transfection with normal cell DNAs¹³. If the viral genome is not required for the maintenance of neoplastic change in LLV-induced tumours, the variability of LLV DNA in the metastatic bursal lymphomas, and the absence of detectable viral DNA in some lymphomas induced by analogous feline retroviruses²¹, is not surprising.

The mechanism of activation of transforming genes in LLV-induced tumours is highly speculative. The pathogenesis of lymphomas induced by LLV suggests a process which includes several pre-neoplastic and neoplastic stages²². In addition, lymphomas pathologically identical to those induced by LLV occur at low frequency in the absence of exogenous LLV infection²³. LLV may act thus at an early pre-neoplastic stage to expand the population of cells in which transforming events occur, which is consistent with the mechanism proposed by McGrath and Weissman²⁴ for induction of T-cell lymphomas by analogous retroviruses of the AKR mouse. LLV infection might also increase the frequency of genetic changes leading to transformation as a result of the activity of enzymes involved in integration of LLV DNA or by packaging transcripts of cellular genes in LLV virions followed by synthesis and integration of homologous DNAs in secondarily infected cells. Finally, the specificity of LLVs for transformation of bursal lymphocytes suggests that these cells provide a preferential site for LLV replication or that a bursal cell population provides a particularly sensitive target for genetic changes leading to transformation.

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Internal mobility of ferrocytochrome c

Scott H. Northrup*§, Michael R. Pear*,
J. Andrew McCammon*||, Martin Karplus†
& Tsunehiro Takano‡

*Department of Chemistry, University of Houston, Houston, Texas 77004

†Department of Chemistry, Harvard University, Cambridge, Massachusetts 02138

‡Department of Chemistry, California Institute of Technology, Pasadena, California 91125

In the refinement of the X-ray diffraction structures of molecules, it is conventional to introduce atomic 'temperature factors' of the Debye-Waller form to characterize the widths of the electron density peaks corresponding to the atoms¹. Although these factors are known to include a variety of contributions other than thermal fluctuations of the atomic positions², recent progress in the refinement of protein structures has led to inferences concerning atomic mobilities from the temperature factor data for several proteins³⁻¹⁰. Atomic position fluctuations can be calculated independently by the molecular dynamics method, in which the classical equations of motion for the atoms of an equilibrated protein are solved on a computer¹¹⁻¹⁶. We now show that the X-ray diffraction and dynamical simulation methods yield similar pictures of the atomic mobility in tuna ferrocytochrome c.

The temperature factors used here are from a new refinement^{17,18} of the X-ray diffraction data collected at 293 K (ref. 19); the final *R*-factor is 17% at a resolution of 1.5 Å. The simulation results are from a new dynamics calculation carried out as described earlier¹⁴, but with the following modification to improve initial equilibration. After the 12 ps of warming from a partially relaxed structure, atomic velocities were randomly re-assigned from a 300 K maxwellian distribution every 0.2 ps for an 8-ps period. The calculation was then continued as before, with 12 ps of unperturbed equilibration followed by 16 ps of actual simulation at 301 K.

The results of the new dynamics calculation are very similar to those found without the additional velocity randomization¹⁴, but the average structure is somewhat closer to the X-ray result and the fluctuations about the average structure are somewhat smaller. Relative to the 1.5-Å X-ray structure¹⁷, the average deviation of C^α positions is 1.27 Å, whereas that for all heavy atoms is 1.54 Å; the corresponding deviations in the previous dynamics calculation¹⁴ are 1.42 Å and 1.73 Å. All these deviations are 0.1-0.2 Å smaller than those relative to the 2.0-Å X-ray structure¹⁹. The r.m.s. fluctuations of the atoms about their average positions are typically 10-20% smaller than in the previous dynamics calculation¹⁴, but the structural correlations are unchanged (see below). In part, this decrease reflects a corresponding decrease in large-scale fluctuations: the r.m.s. fluctuations in radii of gyration are 0.047 Å for C^α atoms and 0.041 Å for all heavy atoms; the corresponding fluctuations in the previous calculation are 0.055 Å and 0.047 Å.

To compare the atomic position fluctuations found in the dynamical simulation with those found in the X-ray diffraction study, it is necessary first to subtract from the atomic temperature factors a contribution due to disorder within the crystal^{3,4}. The atomic temperature factors *B* are given by¹⁻⁴

$$B = (8\pi^2/3)(\langle \Delta r^2 \rangle_{cv} + \langle \Delta r^2 \rangle_{ld}) \quad (1)$$

§ Present address: Department of Chemistry, Tennessee Technological University, Cookeville, Tennessee 38501.

|| To whom reprint requests should be addressed.

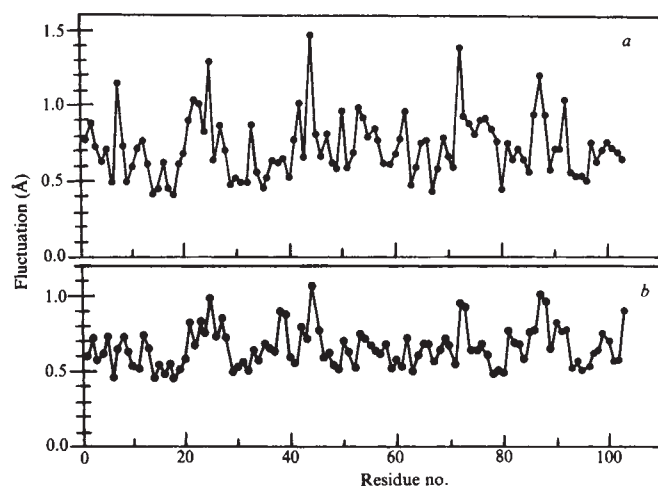


Fig. 1 Average r.m.s. atomic position fluctuation for each residue. *a*, Simulation result; *b*, X-ray result; the experimental values have been corrected uniformly for lattice disorder contributions (see text).

where $\langle \Delta r^2 \rangle_{cv}$ is the mean-square dynamical fluctuation of the atomic position vector r due to conformational and vibrational motion, and $\langle \Delta r^2 \rangle_{ld}$ is the mean-square fluctuation due to lattice disorder. With the simplifying assumption that only translational disorder need be considered, $\langle \Delta r^2 \rangle_{ld}$ is the same for all atoms in the protein⁴. Here, $\langle \Delta r^2 \rangle_{ld}$ was estimated by requiring that the average of $\langle \Delta r^2 \rangle_{cv}$ over all interior atoms (that is, those atoms within 12 Å of the protein centre of mass)¹⁴ obtained from the temperature factors be equal to the corresponding average of the mean-square position fluctuations in the simulation. The resulting value, $\langle \Delta r^2 \rangle_{ld} = 0.22 \text{ Å}^2$, is similar in magnitude to experimental estimates of this quantity for myoglobin crystals ($\langle \Delta r^2 \rangle_{ld} = 3\langle x^2 \rangle_{ld} = 0.14 \text{ Å}^2$)^{3,4}; the somewhat larger value found here is consistent with the fact that the myoglobin estimate is based only on iron atom data. The present comparison suggests that about half of the observed *B* factor value is contributed by thermal motion and half by other effects. In the following discussion, the r.m.s. position fluctuations $\langle \Delta r^2 \rangle_{cv}^{1/2}$ calculated from equation (1) are compared with the r.m.s. position fluctuations from the dynamical simulation.

The magnitudes of the atomic position fluctuations derived from the X-ray temperature factors and from the dynamical simulation show the same correlations with the protein structure as were found in a previous simulation study¹⁴. The average r.m.s. fluctuations of the C α positions are 0.58 Å (X ray) and 0.57 Å (simulation); the corresponding averages for all heavy atoms are 0.66 Å and 0.72 Å. To compare interior and exterior fluctuations, averages were calculated for atoms in spherical shells of 3-Å thickness centred at the protein centre of mass; the results are shown in Table 1. In the interior (shells with outer radii $\leq 12 \text{ Å}$)¹⁴, the fluctuations are relatively small and increase slowly with shell size. In the exterior, the fluctuations increase more rapidly with increasing shell size; larger fluctuations occur in the simulation, reflecting the somewhat higher temperature and the neglect of the protein surroundings in the calculation. The atomic r.m.s. position fluctuations, averaged for each residue, are shown in Fig. 1; similar mobility patterns are evident,

although the surface residues have larger fluctuations in the simulation. The most prominent differences are associated with the large peaks found in the simulation for exterior charged residues (for example, Lys), which are most strongly affected by the crystal and solvent environment.

The haem group is relatively rigid, with average r.m.s. fluctuations of 0.52 Å (X ray) and 0.46 Å (simulation). To assess the effect of the haem group on the mobility of nearby polypeptide atoms, a haem neighbourhood was defined by two cylindrical disks of 6-Å radius and 6-Å thickness lying on either side of the haem and coaxial with the iron¹⁴. The average r.m.s. fluctuations of the 61 heavy atoms in this neighbourhood are 0.54 Å (X ray) and 0.50 Å (simulation), compared with 0.60 Å (X ray) and 0.59 Å (simulation) for the other 374 heavy atoms of the protein interior. The average r.m.s. fluctuations are larger in the disk on the Met 80 side (0.57 Å, X ray; 0.52 Å, simulation) than in the disk on the His 18 side (0.51 Å, X ray; 0.49 Å, simulation). The additional flexibility on the Met 80 side may correlate with the larger structural changes observed on this side on oxidation of the protein¹⁷.

Table 2 Average r.m.s. position fluctuations for various groups of atoms

Group	R.m.s. fluctuations	
	X ray (Å)	Simulation (Å)
C α in helices	0.57	0.54
C α not in helices	0.60	0.59
α atoms*	0.58	0.57
β atoms*	0.63	0.65
γ atoms*	0.72	0.78
δ atoms*	0.76	0.93

* Excluding proline residues.

Comparison of r.m.s. position fluctuation averages for other groups of atoms reveals additional correlations with the protein structure, as is seen in Table 2. In the backbone, there is somewhat less mobility in α -helical regions than in non-helical regions. In the side chains, the mobility tends to increase monotonically with increasing distance from the backbone.

The correlations of atomic mobility with structural features found here are in general agreement with simulation^{11-13,15,16} and X-ray⁴⁻¹⁰ results reported earlier for other proteins (compare with ref. 14). The detailed correspondence between the results from X-ray temperature factors and the molecular dynamics simulation for cytochrome *c* reinforces the view that valid information on the internal mobilities of proteins can be obtained from these complementary approaches.

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Table 1 Average r.m.s. position fluctuations in shells of 3-Å thickness

Outer radius of shell (Å)	No. of atoms in shell	R.m.s. fluctuations	
		X ray (Å)	Simulation (Å)
6	41	0.54	0.51
9	148	0.56	0.53
12	284	0.60	0.61
15	262	0.71	0.83
18	96	0.85	1.09

BOOK REVIEWS

Economics down on the farm

R.C. Lewontin

AGRICULTURAL economists stand out among students of the "dismal science" by being uniquely conscious of history. The paradigm of bourgeois economics is an equilibrium theory that sees the present forms of production and exchange as eternal and without historical roots. Agricultural economists, however, are acutely aware of the rapid changes that have taken place in world agricultural production and in the relations of the agricultural producers to other economic sectors. Their problem is to incorporate these changes into the conventionally accepted explanatory modes of economics and political science that are, by and large, designed to explain the supposed unchanging character of modern capitalism. The explanation invoked combines demographic change, the conflict between interest groups and the effect of technological developments. According to this theory, population growth, especially urban growth, has put increasing pressures on farmers to produce more food. The farmer has responded by adopting labour-saving technology which has resulted in a further displacement of population to cities, a drastic reduction in the relative size of the farm population and, as a consequence, the loss of political power by what was formerly a dominant pressure group in national policies. *Farm and Food Policy* follows this line of analysis and attempts to use it to predict changes in American agriculture during the next few decades.

According to Paarlberg, there have been three major stages in farm policy history (what he calls three "agendas"). The first, ending in 1933, was a period of strong reliance on public funding of research, technology and education in agriculture, by which means the farm sector succeeded in reaping the benefits of technological change that had been created at public expense. The second, ending in the 1960s, was the support of prices paid to the farmer, by means of various government commodity programmes, at the same time holding down farmer costs by exempting the agricultural sector from labour laws, trade laws and certain taxes. Both of these policy agendas were a consequence of the numerical, and therefore political, power of farmers to establish themselves as a unique sector of American business with unique privileges. The loss of this numerical and political power in the 1960s has resulted in the loss of agriculture's unique position and the imposition of a

Farm and Food Policy: Issues of the 1980s. By Don Paarlberg. Pp.338. (University of Nebraska Press: 1980.) \$16.50, £9.90.

third agenda from outside the farm sector. This last agenda supports the interests of farm labour, consumers, conservationists — the non-farm sector at large. Paarlberg's conclusion is that an equilibrium will be reached in the next decades just as the producer-consumer, capital-labour tensions between interest groups have equilibrated in the rest of the economy to the benefit of all parties concerned.

The only trouble with the conventional view of the political history of agriculture in the United States is that it does not correspond very well with what has happened.

First, pure demographic superiority has never meant political power for farmers. In 1786 the farmers of Massachusetts rose in armed rebellion to prevent the sitting of the Courts of Common Pleas which were bringing judgements against them for debt. Farmers comprised over 90% of the population, but the legislature and executive power were in the hands of the merchants and bankers, who put down Shay's Rebellion in a few months and collected what was owed them. There has been no significant period in American history when farmers have succeeded in dominating the state.

Second, the decrease of the farm workforce since 1900, from 40% to 4% of the total labour force, grossly exaggerates the change in the political voice of the agricultural sector. A large part of the relative decrease in farm population is a consequence of the absolute increase in urban population. The non-farm labour force has increased by a factor of five in the USA, while the farm population has fallen by about a factor of three. Much of the reduction in farm labour has been in tenants, sharecroppers and farm labourers who were generally the poorest sector of the farm population and without political power. In 1900, of 5.7 million farms, 2 million were tenancies. By 1970, while there were only 2.7 million farms left, tenants accounted for only 350,000. Moreover, there has been an immense increase in those who service and sell to the farmer — the merchants of farm machinery, of seed and fertilizers, of consumer goods in market towns, the mechanics and servicemen. These are now two to three times as numerous as farmers and are an integral part of the agricultural

sector of the economy. Thus, farmers and those who sell goods and services to them remain numerous and locally concentrated. Despite their lack of economic and political power nationally, the farm sector has, and still does, exert considerable pressure locally where their concentration is high. This local concentration is important because, for example, state funds account for two-thirds to three-quarters of the money spent on agricultural research and education.

Third, agriculture has not been alone in socializing the costs of research by displacing it to public institutions. The degree of the socialization of research costs depends in each case on the point at which such socialization interferes with exclusive private control over the product. The cost of drug research is socialized by having most basic research and chemical trials carried out in public institutions supported by tax money, or by tax-exempt contributions from individuals. Only when the final compounding of the trade-name product is to be done will the drug companies themselves — sometimes — spend research money. Most basic research in metallurgy, chemistry, electronics, transportation, information theory and so on is supported publicly by tax funds or by tax-exempt gifts. The situation is the same in agriculture. Public institutions do the basic breeding research and produce public lines. The seed companies then change them slightly, if at all, give them a brand name and make the profit.

Fourth, it is by no means clear who has received important benefits from socialized research in agriculture. Farmers try to reduce their labour costs, at the same time increasing their control over the labour process. Both these ends have been accomplished by changing from a labour-intensive to a capital-intensive system of production. For farming this has been the result of three major technological changes, none of which was the product of agricultural research. The first, beginning in about 1850 and ending in 1900, was the introduction of labour-saving machinery such as new plough designs, mowers, reapers, harvester combines and the like. All of these were invented and improved by industrial entrepreneurs. Further advance was limited because of the lack of the flexible traction power needed to pull large gang ploughs and combines. Beginning in about 1910 and extending until the Second World War, developments in the automotive industry (gasoline and diesel

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engines, the differential) gave the second great boost to labour productivity in agriculture. Finally, after 1945, the drop in the relative cost of fertilizers and other chemicals led to an immense increase in the use of agricultural chemicals. While none of these changes resulted from agricultural research, they all determined its direction. Plant breeding, for example, is largely devoted to making crop plants more responsive to fertilizers, and easier to harvest mechanically by reducing lodging, increasing morphological uniformity and making ripening simultaneous. The direction of agricultural research is determined by the existing forms of capital investment in production.

Who has benefited from these developments? Paarlberg assumes, conventionally, the consumer. His evidence is that Americans are spending, on the average, a decreasing proportion of their income on food, but this argument is spurious. As every economist (Paarlberg is one) knows, the demand for food, especially in prosperous countries, is virtually insensitive to income, so that as real incomes rise, food necessarily becomes a smaller and smaller proportion of expenditure. The real question is whether the price of food relative to the average price of other commodities has gone down. In 1913, the consumer price index of food relative to all other consumer items was 0.98; in 1967 (the base year) it was 1.00; and in 1979 it was 1.09. So much for cheaper food! Nor is it easy to show that farmers are better off. Paarlberg makes much of the increase in the value of farm real estate, but this is a paper increase that cannot be converted by the farmer into other assets because the market in farm real estate is a very thin one. In fact, the paper inflation of land value is a liability to the farm family because of the immense death duties that must be paid in cash that cannot be realized from continued farm operations. Like other small producers, most farmers consume all their surplus, so to the extent that farmers lead a better material life they have certainly profited. Driving an air-conditioned tractor is clearly better than sweating in the sun behind a mule. Yet the hours are just as long and the risks just as great. On the other hand, there is no doubt at all that the major beneficiaries of the technological revolution in agriculture have been the corporations that provide the farm inputs: chemical, seed and machinery companies, big grain merchants and those who lend money at interest.

Fifth, farmers cannot be treated as a homogeneous interest group sharing certain cultural features that arise from their unique way of life. Paarlberg portrays farmers as conservative, yet agriculturalists have been among the most politically radical groups in America, beyond such uprisings as Shay's Rebellion. In 1912 the Socialist Party had more paid members in Oklahoma than in New York, and Eugene Debs received more than 25% of the vote in

the 23 counties of that state with highest tenancy levels and lowest incomes. The notion that mid-Western farmers are "conservative", despite their long record of populism, comes in large part from their rejection of the internationalist interventionist policies that were of clear advantage to industrial and banking capital and which were the hallmark of 20th century "liberalism". Tenant farmers who made up more than one-third of all American farm families until the Second World War cannot be lumped together with a 600-acre farmer who owns all his land and capital equipment. The political behaviour of farmers at any time reflects not some unique "way of life" but the way each farmer confronts the system of production and exchange of which he is a part.

Far from being unique, the history of agriculture in the United States is, in its main features, the history of American capitalist development. There has been a constant pressure to reduce labour costs and increase control over the labour process, a socialization, where possible, of the costs of technological development, a growth in the size of enterprises, a decrease in their number, and a growing monopoly control over production and exchange. Large corporations do not own the bulk of farmland and are not likely ever to do so because it is a poor use of capital, but that does not mean that agricultural production is any the less under the political and economic control of large capital concentrations. The forms are different, but the substance is the same. □

R.C. Lewontin is a Professor in the Museum of Comparative Zoology and the School of Public Health at Harvard University, and is a member of the World Agricultural Research Group.

NMR for experts

K.A. McLauchlan

NMR of Chemically Exchanging Systems. By Jerome I. Kaplan and Gideon Fraenkel. Pp.165. (Academic: 1980.) £11, \$19.50.

THIS book does not provide an experimental account of rate processes studied by NMR, nor does it give simple recipes for extracting rate data from observations. Instead it constitutes an extensive account of the application of density matrix theory to chemically exchanging systems. Much prior knowledge of NMR theory is assumed, notably of the nuclear spin Hamiltonian, the properties of spin operators and the rotating frame. This approach is on occasions too demanding; for example, some of the important density matrix equations as applied to magnetic resonance are simply quoted rather than derived in their entirety.

To start with, the Bloch equation and

density matrix approaches are shown to yield similar results for a single absorption peak. This early section of the book is the least successful since it abounds with small errors, which make it difficult to follow. Cross-referencing is not always correct and symbols are confused: bold type is used sometimes for vectors and sometimes for operators (which also appear in script and in the text typeface). The density matrix is defined as an operator rather than as a representation of an operator and its usefulness and relevance is not established at an early stage. A student would be better advised to turn to C.P. Slichter's *Principles of Magnetic Resonance* (Springer-Verlag, 2nd edn 1978) for a clearer introduction.

Once into its stride, however, this is an immensely useful and unique book for anyone wishing to see in detail how to apply

the theory to real systems. It covers relaxation theory (including quadrupole effects), chemical reorganizations of all descriptions in first-order and close-coupled situations, the lineshapes of exchanging systems at high and low RF fields, double resonance lineshapes (including with exchange) and, briefly, transient effects. Application of density matrices to 2D spectroscopy receives no attention. Each chapter concludes with a useful summary and bibliography.

This book is intended for "the serious student of NMR" who has a considerable understanding of his subject, and at that level will be of value. □

K.A. McLauchlan is a Lecturer in Physical Chemistry at Oxford University and a Fellow of Hertford College.

A holistic approach to the classification of behaviour

Susan D. and Leslie L. Iversen

Chemical Influences on Behaviour. Edited by K. Brown and S. J. Cooper. Pp.670. (Academic: 1980.) £39.20, \$90.50.

THE editors of this volume have brought together a collection of review articles relating to the effects of 'chemicals' — including hormones, nutrients before and after birth, neurotransmitters and psychotropic drugs — on behaviour. The aim is to provide the student with an introductory discussion of chemical influences on growth and development of the CNS, neuro-endocrinology, behavioural homeostasis and the effects of chemicals on a range of sensory and cognitive functions. The preface states that "the coverage is not intended to be comprehensive but rather serves to introduce the field of chemical influences on behaviour by carefully chosen examples, and to illustrate the continuity in research methods and the common problems of interpretation". Unfortunately this continuity will not be immediately obvious to the student, but it would be a difficult goal to achieve in a text of this kind.

The range of material in the book is unusual, cutting across traditional barriers of specialized interest. Computer simulation of behavioural homeostasis (F.M. Toates) is an unusual bed-fellow for an ethological topic such as chemical communication between animals (K. Brown). Students should find this range of material fascinating. Efforts have been made in some chapters to discuss methodological and other problems in the design of experiments, and Cooper's chapter on drug interaction studies is particularly useful, as the problems of working with drug cocktails and combinations are rarely

discussed.

Three chapters, by Booth, Toates and Blundell, and Latham are dedicated to an analysis of food and water homeostasis. They are all excellent, and when read together illustrate how a combination of research methods applied to a clearly defined problem can yield substantial advances in understanding the organization and control of behaviour.

For the past 50 years studies of eating and drinking have been dominated by efforts to find the brain centres controlling the behaviour. While it is clear that certain hypothalamic and limbic lesions dramatically disrupt such behaviour, these lesion models have, during the past 20 years, contributed little more to our knowledge. Fresh advances have come only relatively recently by paying meticulous attention to the whole physiological balance of the organism, where the brain lies on an axis of control and is not the 'genie in the lamp'. It is useful to compare studies of feeding with those on drinking, where physiologists have always been strongly represented in the research effort. The delicately balanced nature of water control mechanisms has forced experimentalists to consider the homeostatic balance of the whole organism. This approach has paid off handsomely in the elaboration of the extracellular dehydration control of thirst by Fitzsimons (reviewed here by Blundell and Latham). This concept has been extended, most successfully, to investigations of the chemical messengers involved and their sites of action in the brain. Similar research on the control of food intake is poised to follow, as Blundell and Latham illustrate.

Rodgers provides a useful review of aggression from the behavioural, neuro-anatomical and neuropharmacological viewpoint. The relationship between the sensory and emotional responses to noxious stimuli, the pain/anxiety continuum, provides a challenge to research. The catecholamine and indolamine chemical transmitter pathways

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of the hypothalamus and limbic system are involved in the emotional responses of aggression. But these same areas of brain have a high content of endogenous opioid transmitters, the endorphins. The endorphins in the brain stem are involved in the mediation of pain; their existence in forebrain may suggest a physiological axis of pain and anxiety. This is another area of research ripe for exploitation — the behavioural methods for studying responses to pain and aggressive responses are well developed; the chemical and anatomical axes of the spinal cord and brain are defined. It is now timely to ask what the functional relationships are between pain, anxiety and emotional behaviour.

In certain other areas there is less reason for optimism. If we consider the processes of learning and memory, it could be said that we are still struggling for the first rung of the ladder. Whilst it is recognized that there are methodological difficulties in studying memory — these can be overcome with appropriate controls (see Pilcher on state dependency) — there are more serious problems of interpretation: for example, in distinguishing deficits of retrieval from consolidation. We know that certain limbic areas are involved in the processing of information for memory storage, and we know that a wide range of endogenous and exogenous chemicals modify memory. But we still lack insight into the organization of

the normal processes involved.

Much of the information in this volume could be found in a comprehensive textbook of physiological psychology, but there the material would be classified by brain site. The hypothalamus chapter, for example, would include eating, drinking and aggression. The achievement of this volume is that it provides another way of classifying behaviour: on physiological-chemical axes. This is not as novel as it seems; the terms pituitary-gonadal axis and pituitary-adrenal axis, used by Brain in his chapter titles, are traditional concepts in research related to sex hormones and behaviour, and to the relationship between ACTH, steroids and stress. But the same concepts could be applied to all behaviour. The brain and the body are inseparable and each possesses an integrated hierarchy of control. The endogenous neurotransmitter systems of brain and body provide a major force for integration. It is hardly surprising that so many chemicals modify behaviour since neural signalling depends so heavily on chemical relays, and it is with these tools and a conception of the whole organism that we may one day come to understand normal and abnormal behaviour. □

Susan D. Iversen and Leslie L. Iversen are Assistant Director of Research, Department of Experimental Psychology, and Director of the MRC Neurochemical Pharmacology Unit, University of Cambridge.

Asteroid revolution

D.E. Brownlee

Asteroids. Edited by Tom Gehrels. Pp.1181. (University of Arizona Press: 1979.) \$19.95.

THE formation processes which created planets in the Solar System had at least one apparent failure. The failure occurred in the transition zone between the terrestrial and Jovian planets, and the surviving debris is known as the asteroid belt. Planetary formation was aborted at an early stage, presumably due to the proximity and rapid formation of Jupiter, the most massive planet in the Solar System. The majority of asteroids are believed to be composed of early Solar System materials which have been relatively well preserved for the age of the Solar System. They are the major source of meteorites and are a precious resource of information on the origin of the Solar System.

Ten years ago, measurable properties of asteroids were basically limited to orbits, broadband colours and brightness variations. Since then asteroid science has experienced a genuine revolution, with new observational techniques and new ideas producing a wealth of information relating

to the composition and physical nature of asteroids as primitive Solar System bodies. The revolution is documented in this remarkable book, produced and edited by T. Gehrels and M.S. Matthews, with contributions from a significant fraction of the scientists active in asteroid research. The book is the product of a conference held in Tucson, Arizona, but by no means is *Asteroids* an ordinary proceedings volume. Instead of a loosely organized compilation of each participant's research results of the previous six months, this is a carefully created volume composed of review articles which cumulatively provide comprehensive and coherent coverage of existing knowledge of asteroids.

The book contains very few omissions, little repetition but extensive cross-



The Moss Flora of Britain and Ireland by A. J. E. Smith, first published in hardback in 1978, has been issued as a paperback by Cambridge University Press. Fully illustrated with line drawings by Ruth Smith, and containing an artificial key and full descriptions of taxa, the paperback edition costs £12.50.

referencing. Compilation of a 1181 page multi-author book with these attributes is obviously the result of careful planning and detailed iterative editing. Among the topics covered are orbital evolution, collisions, regolith development, reflectance spectroscopy, mineralogy, meteorite properties, formation from the solar nebula and possibilities for exploration by spacecraft.

Reading the volume, one is impressed by the diversity of techniques used to study asteroids. For example, asteroid diameters are now determined by measurement of polarization as a function of phase angle, the ratio of optical and infrared brightness, speckle interferometry, radar and occultations. Prior to 1970 diameters could only be estimated by visual telescopic observation. The most spectacular development described is the application of accurate spectral reflectivity measurements to reveal information on surface mineralogy. The measurements, combined with crystal field theory and laboratory data, have shown that asteroids are complicated bodies with diverse physical properties and surface mineralogy. One of the challenging goals for future theories on the origin of the Solar System will be to explain how such diversity could have been produced in what probably was only a small fraction of the solar nebula. Another exciting aspect of the reflectivity work is the attempt to identify specific asteroid parent bodies for various meteorite types.

Asteroids is a comprehensive and definitive book that will be of value to a variety of readers. For the specialist it will probably be the major reference source

until future developments add a major increment to existing knowledge; such an advance will possibly not occur until a multi-asteroid spacecraft mission can be flown. For the newcomer to asteroid work the book will be invaluable because it contains numerous suggestions for future research. It also contains 140 pages of tabulated data for a large number of asteroids; this remarkable compilation contains information on orbital parameters, spectral reflectances, polarimetry, radiometry, light curves, magnitudes, diameters and discovery circumstances. In addition to active researchers, *Asteroids* will appeal to a much wider audience because of the review nature of the contributions. To smooth the mismatches that often occur in such an interdisciplinary field, a glossary is also included.

Although this is an excellent book it does, of course, have some shortcomings. Fortunately, these are of a relatively minor nature. A mineral defined in the glossary is misspelled and a few of the glossary definitions are not well thought out. In the text some of the material on meteorites is not as comprehensive as it could have been and does not reveal to the general reader the elements of controversy that exist in certain areas. In almost all other aspects, however, *Asteroids* is a real gem and remarkably enough for a book of its size is inexpensive enough to be a painless addition to one's personal book collection.

D.E. Brownlee is Associate Professor of Astronomy at the University of Washington, Seattle, and Associate in Geochemistry at the California Institute of Technology, Pasadena.

Children's skills with a difference

Peter Bryant

Beyond Universals in Cognitive Development. By David Henry Feldman. Pp.204. (Ablex: New Jersey, 1980.) \$17.50

THERE is a character in Flaubert's short tale *A Simple Life*, who on being shown a map of America where her adopted son was at the time asked if she could see his house on the map and then if she could see him. Here is a dramatic if extreme illustration of the fact that people's skills with maps vary widely. This sort of variation between people is David Feldman's starting point and maps happen to be his main example.

His basic argument is impeccable. People's skills vary wildly. Some people play chess well, others are excellent gardeners and so on. Yet studies of the development of skills in children have concentrated on the few which virtually all adults possess. The understanding of

number, the ability to make and understand logical inferences, even reading and writing are achievements which we take for granted in other adults, and yet these are at the centre of studies of child development. We spend very little time on studying how adults become so different from each other.

Feldman's approach is to look for similar variability among children, and he looks at two topics. The first is the way in which children draw maps. He divides this skill into a number of components and shows that different children forge ahead with different components. So much seems to be empirical fact. What causes this variability is still a matter for speculation, of which there is a great deal in this book.

His second example is creativity, but here because the measures of this elusive quality have been so unsuccessful Feldman's account is much less impressive. But he is right to point out that we have concentrated too much on universal features on human development and too little on the variations. □

Peter Bryant is Watts Professor of Psychology at the University of Oxford.

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